RAD
Croatian Academy of Sciences and Arts - Medical Sciences

Editorial Board
Uredništvo

Vol 567 = 70-71 (2025)
knjiga 567, svezak 70-71

doi 10.21857/y6zolb3l1m
issn 1848-641x (online)
issn: 1330-5301 (print)

Publisher
Izdavač
Croatian Academy of Sciences and Arts
Department of Medical Sciences
Hrvatska akademija znanosti i umjetnosti
Razred za medicinske znanosti

For the Publisher
Za izdavača
Dario Vretenar

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Filip Đerke

Graphic designer / Grafička urednica
Nina Ivanović

Edition / Naklada
100

DOI numbers / DOI brojevi
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RAD CASA Medical Sciences is published twice a year as a double issue.

RAD CASA Medical Sciences is included in / indexed by:

Printed with support of the Foundation of the Croatian Academy of Sciences and Arts

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RAD
Croatian Academy of Sciences and Arts - Medical Sciences

Vol 567 = 70-71 (2025)
knjiga 567, svezak 70-71

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HRVATSKE AKADEMIJE ZNANOSTI I UMJETNOSTI
MEDICINSKE ZNANOSTI

VOL 567 = 70-71 (2025)
KNJIGA 567, SVEZAK 70-71

SADRŽAJ

Analysis of Medical Students' Success Performing Endotracheal Intubation (ETI) and Predictive Model for Success Probability

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ABSTRACT

Introduction: Endotracheal intubation (ETI) is a critical skill every medical student should master during their education, at least on the basic level. Today, approaches in training and evaluating medical students regarding ETI vary, each with its benefits and drawbacks. The aim of this analysis was to comparatively evaluate different components of medical students' education regarding ETI.

Materials and methods: From the PubMed database, we selected 16 studies that reported on medical student ETI success rates with various training and evaluation techniques. The variables observed were time period of study, evaluation setting, evaluation technique, and training type, while the primary outcome was average intubation success rate (ASR).

Results: The analysis included 1610 medical students. A statistically significant superior score was noted in the indirect laryngoscopy subgroup compared to direct laryngoscopy, the mannequin model subgroup compared to operating room patients, and the standard training subgroup compared to advanced simulator training. Additionally, the trend of ASR change from the 1980s to today was shown to be significantly positive. Finally, as a form of result validation, we used machine learning modelling for intubation success prediction. Based on the provided variables, the model had a > 90% accuracy in predicting which student might have a higher than 70% probability of success.

Conclusions: In conclusion, this study demonstrates a significant improvement in student intubation skills over time, as well as a clear superiority of indirect laryngoscopy scores, and provides a contribution to the determination of an optimal educational program for ETI skill acquisition.

Keywords: Artificial intelligence; Intubation, Intratracheal; Students, Medical

Sažetak:

Analiza uspjeha studenata medicine u provođenju endotrahealne intubacije (ETI) i prediktivni model vjerojatnosti uspjeha

Uvod: Endotrahealna intubacija (ETI) ključna je vještina koju bi svaki student medicine trebao savladati tijekom svog obrazovanja, barem na osnovnoj razini. Danas se pristupi u obuci i evaluaciji studenata medicine u vezi s ETI razlikuju, a svaki ima svoje prednosti i nedostatke. Cilj ove analize bio je usporedno procijeniti različite komponente obrazovanja studenata medicine u vezi s ETI. Materijali i metode: Iz baze podataka PubMed odabrali smo 16 studija koje su izvještavale o stopama uspjeha ETI studenata medicine s različitim tehnikama obuke i evaluacije. Promatrane varijable bile su vremensko razdoblje studija, okruženje evaluacije, tehnika evaluacije i vrsta obuke, dok je primarni ishod

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This article was submitted to RAD CASA - Medical Sciences as the original article

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 18 April 2025
Accepted: 29 May 2025
Published: 25 June 2025

Citation:
Brenner E, Bulić L, Vrbanović Mijatović V. Analysis of Medical Students' Success Performing Endotracheal Intubation (ETI) and Predictive Model for Success Probability
567=70-71 (2025): 8-17
DOI: 10.21857/90836c2zly

bila prosječna stopa uspjeha intubacije (ASR). Rezultati: Analiza je obuhvatila 1610 studenata medicine. Statistički značajno superiorniji rezultat zabilježen je u podskupini s indirektnom laringoskopijom u usporedbi s izravnom laringoskopijom, podskupini s modelom lutke u usporedbi s pacijentima u operacijskoj sali i podskupini sa standardnom obukom u usporedbi s naprednom obukom na simulatoru. Osim toga, pokazalo se da je trend promjene ASR-a od 1980-ih do danas značajno pozitivan. Konačno, kao oblik validacije rezultata, koristili smo modeliranje strojnog učenja za predviđanje uspjeha intubacije. Na temelju navedenih varijabli, model je imao točnost > 90% u predviđanju koji bi student mogao imati vjerojatnost uspjeha veću od 70%.
Zaključci: Zaključno, ova studija pokazuje značajno poboljšanje vještina intubacije studenata tijekom vremena, kao i jasnu superiornost rezultata indirektne laringoskopije te doprinosi određivanju optimalnog obrazovnog programa za stjecanje vještina intubacije.

Ključne riječi: Umjetna inteligencija; Intubacija, Intratrahealna; Studenti, Medicina

Introduction

A frequent preoperative procedure that can pose a challenge to many medical students and young physicians is endotracheal intubation (ETI). While this procedure is commonly performed, mistakes in its execution are costly and can even cause life-threatening complications for the patient. These include trauma, infection, hypoxia, hematoma, etc[1].
Many studies have deliberated on the best way to aid medical students in mastering this technique, as well as on how to best evaluate them. The long-term aim of these studies was to reduce complications regarding ETI, by devising an optimal educational protocol for clinical-year medical students. With the revolution of augmented reality and virtual reality technologies, this has been taken further than ever before. Students can now be taught and assessed in a virtual space and practice repeatedly with no limitations, an excellent example of which are the 'smart glasses'[2]. Studies centred around this topic have often performed these assessments comparatively, usually with different groups of medical students or with groups of residents and even physicians who have completed residency[3]. Another aspect of comparison is between different instruments and techniques of ETI, such as the Macintosh laryngoscope or the flexible fiberscope[4].
The aim of this analysis was to compare different training and assessment techniques for medical students' skills and success in performing ETI. By interpretation of the results, we hoped to discover and highlight the optimal training and assessment methods that might, in the future, result in physicians with a lesser number of complications.

Materials and Methods

An extensive search of the PubMed database was conducted using the keywords "medical students" and "intubation skill". There was no specific range regarding the year of publication. The search yielded 208 results which included all types of scientific publications. All types of literature except original studies and meta-analyses were excluded from the analysis, leaving 42 results in the search. Each of the studies was analysed individually and only those with clearly defined objective student intubation success rates were taken into consideration (Figure 1). In the end, 16 scientific papers were included in the analysis[5-20].
The observed parameters were the time period of study conduction (or publication if not otherwise stated), number of students enrolled, evaluation setting (operating room patients vs mannequin intubation model), evaluation technique (direct laryngoscopy vs indirect laryngoscopy), type of previous training (standard training vs advanced simulator training), and average success rate (ASR). The ASR was considered to be the primary outcome for comparative statistical analysis. Statistical analysis was performed by the JASP statistical software (JASP Team (2024). JASP (Version 0.18.3)[Computer software]). Distribution differences between specific groups were evaluated using the Welch t-test. Distribution differences between time periods were evaluated using the Kruskal-Wallis test. The level of statistical significance was determined at $\alpha = 0.05$. The average success rate of each study was weighted according to the number of students enrolled. Studies that contained separate cohorts based on any of the observed parameters were treated as separate populations and added to their respective categories. In the case of comparative crossover studies, each student was counted once for each cohort they were part of at some point.
Based on the four independent variables (time period, evaluation setting, evaluation technique, and type of training), we trained a machine learning model for intubation success prediction. All

entities in the database with an average success rate of 0.7 or over were classified as the "over 0.7" class, while the rest were classified as "under 0.7". Considering predictions were made for individual entities, this value was no longer interpreted as an average success rate but rather as a derived intubation success probability. Having reduced the problem to a binary classification, we used the RandomForestClassifier algorithm. The model was trained and tested using 10-fold cross-validation. Model quality was evaluated based on accuracy, precision, recall, F-score, and confusion matrix analysis, with "over 0.7" regarded as the positive result. The WEKA workbench was used for model training and evaluation (Eibe Frank, Mark A. Hall, and Ian H. Witten (2016). The WEKA Workbench (Version 3.8.6)[Computer software]).

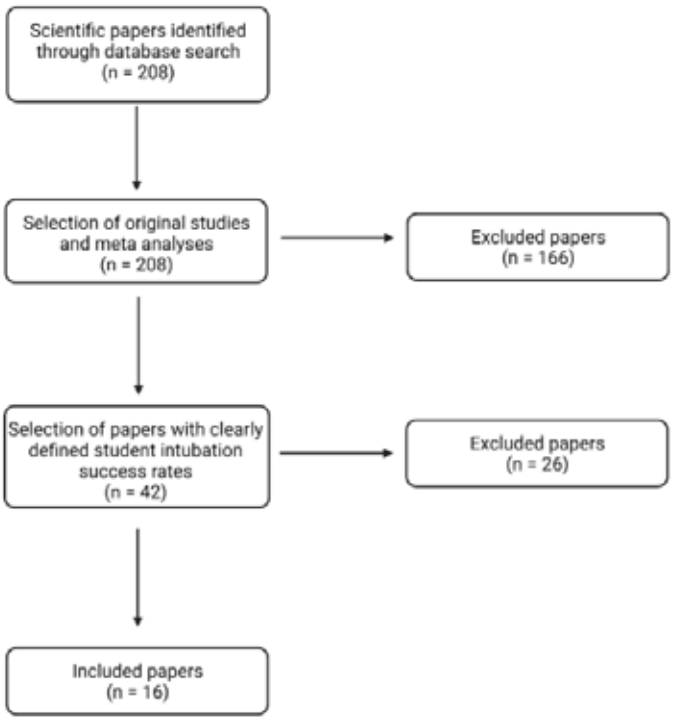

Figure 1. Study selection chart

Results

The selected papers provided a total of 1610 students for analysis. Using descriptive statistics, the distribution of students was determined for each category (Table 1). The Kolmogorov-Smirnov test for the distribution normality regarding ASR produced a p-value < 0.001, indicating a non-normal distribution (Figure 2).

After the initial analysis, comparative statistics for each category were done based on the ASR variable. The first observed comparison was between students tested by direct laryngoscopy and those tested by indirect laryngoscopy (Table 2). When observing the categories in total, there was a statistically significant difference between the ASR scores ($p < 0.001$), with indirect laryngoscopy being superior. The comparison could not be made among the students who were evaluated on operating room patients, while a statistically significant difference was noted in the mannequin intubation model subgroup ($p < 0.001$). When observing the type of training students had received, a difference was seen among students with advanced simulator training ($p < 0.001$) and students with standard training ($p < 0.001$). Both had a significantly superior score on the indirect laryngoscopy method. Regarding the year of study, the comparison could not be made in the period of 1981-2000. From 2001 to 2015, students showed superior success rates in using indirect laryngoscopy ($p < 0.001$) and the same result was noted in the period 2016 to today ($p < 0.001$).

The second observed comparison was the type of training students had received beforehand (Table 3). When observing the differences based on training, standard training in total yielded significantly better scores ($p < 0.001$). Statistically significant differences were in the mannequin intubation model ($p < 0.001$), while a comparison of operating room patients could not be done. When compared based on the evaluation method, students demonstrated significantly superior success rates in standard training with direct laryngoscopy ($p < 0.001$) and indirect laryngoscopy ($p < 0.001$). Finally, in the time periods 2001-2015 and 2016-today, standard training yielded significantly superior success rates ($p < 0.001$).

Thirdly, we observed differences between students who performed intubation on the mannequin model and operating room patients (Table 4). The general comparison and comparison in the standard training group demonstrated a significantly lesser success rate in operating room patient intubation than in the mannequin model ($p < 0.001$). The ASR means in the direct laryngoscopy group were equal and the difference was statistically insignificant ($p = 0.649$). Regarding time period, the mannequin model group had a significantly superior score in the 1981-2000 period ($p < 0.001$), while the operating room patient group had a significantly superior score in the 2001-2015 period ($p < 0.001$).

Table 1. Distribution of students for each variable

CATEGORY	NUMBER OF STUDENTS	PERCENTAGE
Total	1610	100%
Evaluation setting		
Mannequin intubation model	1307	81.2%
Operating room patients	303	18.8%
Evaluation technique		
Direct laryngoscopy	1312	81.5%
Indirect laryngoscopy	298	18.5%
Training type		
Standard training	1248	77.5%
Advanced simulator training	362	22.5%
Year		
1981-2000	95	5.9%
2001-2015	1002	62.2%
2016-today	513	31.9%
CATEGORY	**MEAN**	**STANDARD DEVIATION**
Average success rate	0.76	0.16

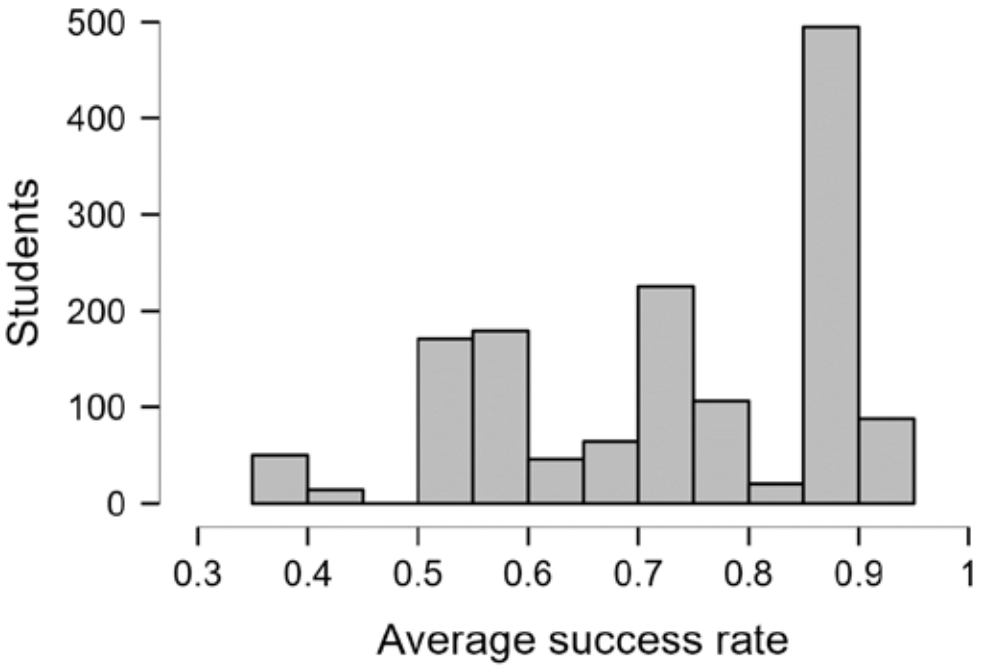

Figure 2. Distribution of ASR

Table 2. Comparison of mean ASR between direct laryngoscopy and indirect laryngoscopy

Stratification	Mean ASR		p-value*
	Direct laryngoscopy	Indirect laryngoscopy	
Total	0.74	0.85	**<0.001**
Evaluation setting			
Operating room patients	0.74	N/A	N/A
Mannequin intubation model	0.74	0.85	**<0.001**
Training type			
Standard training	0.80	0.87	**<0.001**
Advanced simulator training	0.58	0.72	**<0.001**
Year			
1981-2000	0.67	N/A	N/A
2001-2015	0.69	0.80	**<0.001**
2016-today	0.87	0.91	**<0.001**

**Welch t-test; N/A - not available*

Table 3. Comparison of mean ASR between standard training and advanced simulator training

Stratification	Mean ASR		p-value*
	Standard training	Advanced simulator training	
Total	0.81	0.59	**<0.001**
Evaluation setting			
Operating room patients	0.74	N/A	N/A
Mannequin intubation model	0.83	0.59	**<0.001**
Evaluation method			
Direct laryngoscopy	0.80	0.58	**<0.001**
Indirect laryngoscopy	0.87	0.72	**<0.001**
Year			
1981-2000	0.67	N/A	N/A
2001-2015	0.77	0.60	**<0.001**
2016-today	0.89	0.43	**<0.001**

**Welch t-test; N/A - not available*

Table 4. Comparison of mean ASR between mannequin model and operating room patient intubation

Stratification	Mean ASR		p-value*
	Mannequin intubation model	Operating room patients	
Total	0.77	0.74	**<0.001**
Training type			
Standard training	0.83	0.74	**<0.001**
Advanced simulator training	0.59	N/A	N/A
Evaluation method			
Direct laryngoscopy	0.74	0.74	0.649
Indirect laryngoscopy	0.85	N/A	N/A
Year			
1981-2000	0.70	0.61	**<0.001**
2001-2015	0.69	0.76	**<0.001**
2016-today	0.88	N/A	N/A

**Welch t-test; N/A - not available*

Finally, the statistical difference in ASR between the three time periods was analysed (Figure 3). There was a statistically significant difference in ASR between the three time periods ($p < 0.001$). Analysis revealed that the ASR has been increasing over time, from the 0.65-0.7 range in the 1980s and 1990s, to the 0.85-0.95 range today.

The machine learning model for the prediction of intubation success probability was successfully trained using our cohort and the RandomForestClassifier. The overall predictive accuracy of the model was 90.93%, with a precision of 0.905, recall of 0.976, and F-score of 0.939. The confusion matrix showed the true positive, true negative, false positive, and false negative absolute counts (Table 5).

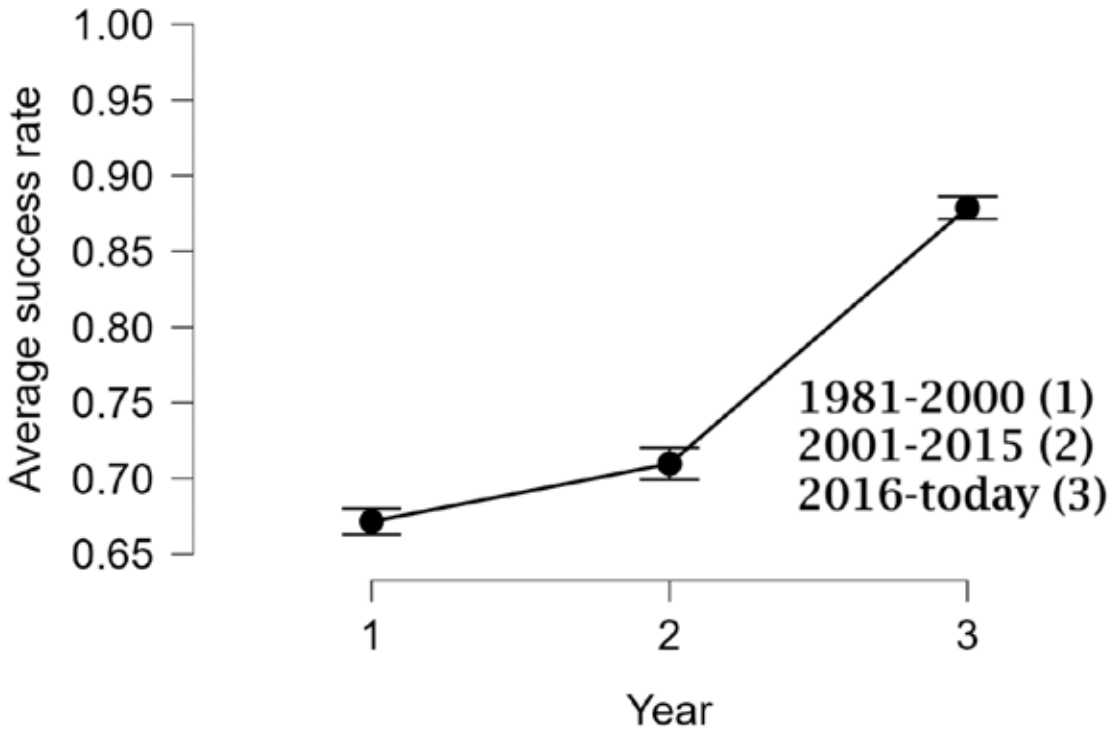

Figure 3. Distribution analysis of ASR in different time periods

Table 5. Predictive model confusion matrix for "over 0.7" class

Predicted value / True value	**Over 0.7**	**Under 0.7**
Over 0.7	1121	28
Under 0.7	118	343

Discussion

Our analysis was conducted on a population of 1610 medical students who were evaluated on endotracheal intubation skills after receiving previous training. Statistical analysis showed many significant differences between different groups of students based on the observed categories.

When discussing the selected evaluation method, the two in question were direct and indirect laryngoscopy. In total, as well as in all observed stratifications, students had better success scores when tested on the indirect laryngoscopy method. The difference between the total ASR for direct and indirect laryngoscopy had a difference of $\Delta = 0.11$. When analysing the studies of our selected pool which specifically observed differences between direct and indirect laryngoscopy, the range of success range differences was $\Delta \in \langle 0.0\text{-}0.2 \rangle$. The indirect laryngoscopy scores were equal to or superior to the direct laryngoscopy scores in all cases but one. In the study by Aghamohammadi H et al., the number of successful intubations was identical in all testing iterations but one, in which the direct laryngoscopy group had two more successful students[13]. However, it is worth noting that the study also greatly focused on intubation times and demonstrated that the intubation time results were significantly superior in the indirect laryngoscopy group. The following results greatly indicate that medical students perform better when evaluated using the indirect laryngoscopy method, rather than direct laryngoscopy. A study published by Prekker ME et al. further supports this finding[21]. The authors made the comparison on clinicians and critically ill patients in multiple medical centres. Video laryngoscopy provided a significantly greater number of successful intubations on the first attempt. Our results demonstrate an increase in ASR over time in both direct and indirect laryngoscopy groups. The ASR is especially high in the indirect laryngoscopy group in the most recent time period, reaching up to 94%. These trends might follow the evolution of intubation technologies which are becoming easier and easier to handle.

When discussing the training the students received before evaluation, we compared the success after training in advanced simulator settings to standard training modes. The students with standard training had a generally significantly higher ASR in comparison to the students trained with advanced simulators ($\Delta = 0.22$). The superiority of standard training was demonstrated in all compared subcategories. The advanced simulator training category involved high-fidelity systems with or without continuous quantitative feedback, complex fully equipped *in situ* simulations, and virtual computer programs for fibreoptic intubation. The two studies using *in situ* simulations (Minai F et al.) and virtual intubation programs (Boet S et al.) demonstrated a significantly superior score with this type of training in comparison to the standard group[19,20]. Furthermore, the scores of students trained with advanced systems were individually high (>80% for acceptable intubation). On the other hand, two studies comparatively used high-fidelity simulators with or without quantitative feedback. In the study by Hempel G et al., the training impact of high-fidelity simulators was compared with and without quantitative feedback[14]. The success rates of correct positioning of the endotracheal tube upon examination for both groups were in the 50%-60% range, but a slightly better score was noted in the group with additional quantitative feedback. Finally, the study by Yau SY et al. involved high-fidelity simulators for the training and evaluation of multiple groups, including medical students and clinicians[9]. Only the medical student group was taken into account in the analysis and their success rate was in the 40%-50% range. While the results in this analysis demonstrated a superiority of success rates following standard training methods, the great potential and utility of advanced technologies in this regard cannot be overlooked. A review by Duffy CC et al. emphasizes the importance of virtual reality in airway management training, stating the need for consensus and guidelines on the incorporation of these technologies into modern curriculums[22]. One important matter that must be kept in mind as simulation technologies advance is their fidelity to real-life situations, as training in unrealistically optimal conditions may negatively impact performance in real-life scenarios. On the other hand, as intubation technologies themselves are rapidly advancing, real-life intubation scenarios are also decreasing in difficulty (best demonstrated by the success rates in direct and indirect laryngoscopy).

When discussing the selected evaluation setting, we compared the evaluations performed on the mannequin intubation model and operating room patients. The ASR was significantly superior in the mannequin group when compared to the preoperative patient group ($\Delta = 0.03$). This trend was also noted in the subgroup comparisons, with two exceptions. Upon exclusion of the indirect laryngoscopy students, the difference between evaluation settings was insignificant. Secondly, when comparing only the time period 2001-2015, the preoperative patient group had higher ASR. Our analysis included no comparative studies which focused on this aspect of research. The studies which solely observed medical student intubation skills on preoperative patients reported success rates in the 60%-80% range[5,7,10]. This cohort of students had little diversity, as there were no students with advanced simulator training or students evaluated using indirect laryngoscopy in this cohort. A point that must be considered when evaluating students on mannequins is how this will translate into intubation skills in actual patients. To investigate this very question, a study was conducted by Lubin J et Carter R[23]. The authors concluded that daily practice on airway management mannequins did not translate into improved clinical performance regarding intubation.

Finally, the trend of general intubation success score was analysed over different time periods. While the score has consistently increased with time, a drastic difference can be noted between

the 1981-2000 and 2001-2015 periods on one hand and the 2016-today period on the other. This might suggest that medical students are becoming more successful in acquiring intubation skills and successfully completing evaluations as technology evolves. In their study, Lin YF et al. analysed how the use of smart glasses impacted students' satisfaction with training and self-assessment of efficacy[2]. In their conclusion, the authors stated that both of these variables were positively impacted on account of implementing advanced technology, a fact that highlights the importance of continued development and utilization of simulation training technologies.

Machine learning predictive model training based on the provided data yielded excellent results, with a high accuracy of > 90%. Students from studies with average success rates over 0.7 were identified efficiently, while those from studies with average success rates under 0.7 were identified with a little more difficulty. This additional result not only serves as a positive mark of data quality but opens the idea for implementation of machine learning prediction for student intubation success probability. The practical use of such models is a thoughtworthy idea that should be explored in future studies in this field.

Limitations

The limitations of this analysis are significant and must be taken into account upon result interpretation. Firstly, the inclusion criteria in terms of study participants was that the participants were required to be medical students. Further than this criterion, the population variables were not controlled. For this reason, it is possible that different backgrounds, educations, nationalities, ages, etc. impacted certain differences in ASR between groups. Secondly, the ASR for each study was not always a product of the same procedures. Some studies tested students in multiple iterations and calculated the average success rate as an average of all attempts, while other studies gave students only one evaluation. Furthermore, the term 'success' was not uniquely defined, but rather varied from physically successful intubation to passing grade upon evaluation (this took into account other performance components aside from successfully positioning the device into the airway). Thirdly, the groups of standard and advanced simulation training proved difficult to define. The advanced simulation training group consisted of technologies that the authors emphasized in their studies, such as high-fidelity simulators and *in situ* complex operating room simulations. Other modes of learning were categorized into the standard training group, creating a great variance in what the term "standard" encompassed. In general, fitting the greatly heterogeneous data we had at our disposal into a homogenous categorical system for statistical analysis proved difficult. As for machine learning model training, the amount of bias and overfitting introduced with the accumulated data is questionable. The high-accuracy result that was achieved might be further evaluated on other datasets with the analysed variables.

Conclusions

Endotracheal intubation is one of the key procedures of anaesthesiology and emergency medicine and requires great skill and training. In order to secure the best performance of this procedure from healthcare providers, a consensus must be reached on the characteristics of an optimal curriculum. For the purpose of educating medical students, sophisticated airway management mannequins have been made. Furthermore, advanced technologies are being developed for training purposes, including high-level feedback simulators and virtual reality programs. Additionally, modern tools for easier intubation are also being developed for clinical but also educational use. This analysis provides a breakdown of the impact these components have on student intubation skills and hopes to contribute to the development of the aforementioned education guidelines.

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Hypertriglyceridemia: From an Innocent Bystander to an Independent Cardiovascular Risk Factor in Young Prehypertensive Subjects

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This article was submitted to RAD CASA - Medical Sciences as the original article

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 12 May 2025
Accepted: 29 May 2025
Published: 25 June 2025

Citation:
Josipović J, Jelaković A, Valent Morić B, Prelević V, Mirošević G, Bulj N, Pećin I, Matijaca H, Ivanko I, Gabrić ID, Reiner Ž, Jelaković B. Hypertriglyceridemia: from an innocent bystander to an independent cardiovascular risk factor in young prehypertensive subjects 567=70-71 (2025): 18-26
DOI: 10.21857/m8vqrt3gp9

Abstract

Introduction: Prehypertension is increasingly recognized as a state associated with adverse cardiometabolic profiles, even in apparently healthy individuals. Although triglycerides (TG) have been implicated in cardiovascular disease (CVD), their role in low-risk prehypertensive populations remains underexplored.

Aim: To compare the cardiorenometabolic characteristics and cardiovascular risk (CVR) of prehypertensive (PHT) individuals with normotensive (NT) and hypertensive (HT) counterparts, with a focus on the independent contribution of TG.

Materials and Methods: A cross-sectional study included 323 untreated adults aged 18–45. Participants underwent anthropometric assessments, standardized blood pressure measurements, fasting blood sampling, spot urine collection, and transthoracic echocardiography. CVR was estimated using the Framingham 10-Year General Cardiovascular Risk Score.

Results: Compared to the NT group, PHT individuals had significantly higher BMI, waist circumference, HOMA-IR, LDL-cholesterol, TG, uric acid, and left ventricular mass index (LVMI) ($p<0.05$). No differences were observed in renal function markers. A stepwise increase in CVR across BP categories was evident (7.9% NT vs. 24.6% PHT vs. 48.1% HT; $p<0.05$). Multivariate logistic regression identified TG as an independent predictor of CVR in the PHT group (Exp(β)=1.88, 95% CI 1.08–3.28, p=0.03).

Conclusion: Hypertriglyceridemia is an early and independent marker of cardiovascular risk in young, prehypertensive individuals. Early identification may improve risk stratification and guide preventive strategies.

Keywords: Prehypertension; Blood Pressure; Hypertension; Hypertriglyceridemia; Risk Factors

Sažetak

Hipertrigliceridemija; Od nevinog promatrača do neovisnog faktora kardiovaskularnog rizika kod mladih predhipertenzivnih osoba

Uvod: Prehipertenzija se sve više prepoznaje kao stanje povezano s nepovoljnim kardiometaboličkim profilima, čak i kod naizgled zdravih osoba. Iako su trigliceridi (TG) povezani s kardiovaskularnim bolestima (KVB), njihova uloga u populacijama s niskim rizikom od prehipertenzije ostaje nedovoljno istražena.
Cilj: Usporediti kardiorenometaboličke karakteristike i kardiovaskularni rizik (KVR) prehipertenzivnih (PHT) osoba s normotenzivnim (NT) i hipertenzivnim (HT) osobama, s naglaskom na neovisni doprinos TG.
Materijali i metode: Presječna studija obuhvatila je 323 neliječene odrasle osobe u dobi od 18 do 45 godina. Sudionici su podvrgnuti antropometrijskim procjenama, standardiziranim mjerenjima krvnog tlaka, uzimanju uzoraka krvi natašte, prikupljanju urina na trenutak i transtorakalnoj ehokardiografiji. KVR je procijenjen pomoću Framingham 10-godišnjeg općeg kardiovaskularnog rizika.
Rezultati: U usporedbi s NT skupinom, osobe s PHT-om imale su značajno veći BMI, opseg struka, HOMA-IR, LDL-kolesterol, TG, mokraćnu kiselinu i indeks mase lijeve klijetke (LVMI) ($p<0,05$). Nisu uočene razlike u markerima bubrežne funkcije. Postupni porast CVR-a u svim kategorijama krvnog tlaka bio je evidentan (7,9% NT u odnosu na 24,6% PHT u odnosu na 48,1% HT; $p<0,05$). Multivarijantna logistička regresija identificirala je TG kao neovisni prediktor CVR-a u PHT skupini (Exp(β)=1,88, 95% CI 1,08–3,28, $p=0,03$).
Zaključak: Hipertrigliceridemija je rani i neovisni marker kardiovaskularnog rizika kod mladih osoba s prehipertenzijom. Rana identifikacija može poboljšati stratifikaciju rizika i usmjeriti preventivne strategije.

Ključne riječi: Prehipertenzija; Krvni tlak; Hipertenzija; Hipertrigliceridemija; Čimbenici rizika

Introduction

Prehypertension (PHT) has long been associated with an increased risk of developing hypertension (HT), cardiovascular diseases (CVD), and premature mortality, even before it was formally defined in the JNC-7 guidelines as a distinct blood pressure (BP) category (1–4). Data from the Framingham Heart Study showed that individuals with high–normal BP had significantly elevated risk of CVD compared to those with optimal BP, with hazard ratios of 2.5 in women and 1.6 in men, both adjusted for standard CV risk factors (2). A large meta-analysis involving nearly one million participants confirmed a linear association between BP and CV mortality, with risk doubling for every 20 mmHg rise in systolic BP and 10 mmHg rise in diastolic BP, beginning at levels as low as 115/75 mmHg (3). Definitions of PHT vary across guidelines. The JNC-7 classification encompasses systolic BP of 120–139 mmHg or diastolic BP of 80–89 mmHg, whereas the 2007 ESC/ESH and later European guidelines divide this range into "normal" (120–129/80–84 mmHg) and "high–normal" (130–139/85–89 mmHg) categories. In contrast, the 2017 ACC/AHA guidelines now consider BP values within the 130–139/80–89 mmHg range as stage 1 hypertension (4–9). These inconsistencies reflect the lack of consensus on defining "normal" BP and the benefits of early, intensive management. Numerous studies in both Western and Asian populations have demonstrated that PHT is linked with adverse cardiometabolic profiles, even among individuals who are otherwise healthy (10–12). The concept of PHT was introduced to identify individuals with above-average CV risk in whom preventive measures could delay or prevent the development of full-blown HT and related complications (13).
This study aimed to assess the clinical and metabolic profiles with the focus on hypertiglycerdiemia of young, untreated PHT individuals in to normotensive (NT) and untreated HT participants, focusing on their association of BP with CVD risk.

Materials and Methods

Subjects A total of 326 participants aged 18–45 years of both sexes were included in this cross-sectional study. Of these, 175 were selected from the hypertension, nephrology and cardiology outpatient clinics of University Hospital Centre Sestre milosrdnice or were voluntary employees of the same institution, based on anamnesis indicating either previously measured normal, high-normal BP or newly diagnosed, untreated HT. The remaining 151 participants were selected from the cohort of the research project "Endemic Nephropathy in Croatia – Epidemiology, Diagnostics, and Etiopathogenesis" (project code: 108-0000000-0329), funded by the Ministry of Health of the Republic of Croatia, based on satisfactory inclusion criteria. The study was conducted at the Department of Internal Medicine of University Hospital Centre Sestre milosrdnice and in the field

within Brodsko-posavska County. Data collection was conducted between April 2016 and April 2018. Participants were excluded if they met any of the following criteria: age below 18 years, BP values outside the defined range (BP >160/100 mmHg), diabetes mellitus, cardiac arrhythmias, heart failure (NYHA class I), coronary artery disease, chronic kidney disease (eGFR CKD-EPI <60 mL/min/1.73 m²), limb amputation, acute inflammation, use of antihypertensive medication, nonsteroidal anti-inflammatory drugs, steroids, oral contraceptives, pregnancy and breastfeeding, and lack of signed informed consent. All participants were interviewed using a pre-structured questionnaire covering family and personal medical history, medication use, and smoking habits. The study complied with the Declaration of Helsinki and local institutional guidelines. Ethical approval was obtained from relevant committees, and all participants provided written informed consent.

Clinical and Laboratory Assessments All measurements were performed in the morning between 08:00 and 09:30 after an overnight fast. Anthropometric data were collected using standardized protocols. Blood pressure was measured in the sitting position using an automated Omron M6 device, with a cuff appropriate to arm size, following a 5-minute rest. BP was recorded in mmHg and measured three times to minimize bias (6). Fasting venous blood samples (10.5 mL) and spot urine samples were collected from all participants. The complete blood count was determined via laser scattering technology (analyzer XN 1000, Sysmex); the biochemistry panel was obtained after 10 minutes of blood centrifugation. The fasting blood glucose was assessed via UV photometry with hexokinase (Architect analyzer), triglycerides via photometry with glycerol phosphate oxidase (GPOPAP), HDL-cholesterol via the homogeneous enzyme-immunoinhibitory method, LDL-cholesterol via the homogeneous method (CHE, CHOD-DSBmT), and C-reactive protein via the immunoturbidimetric method with latex particles. The serum urate levels were assessed via the spectrophotometric uricase enzyme-based method, serum insulin levels via electrochemiluminescent immunoassay (ECLIA, Cobas e 411, Roche), serum and urinary creatinine levels via photometry with alkaline picrate (Architect analyzer, Abbott), serum and urine electrolytes via the indirect potentiometric method, and urine alpha-1-microglobulin via continuous photometry with alkaline picrate and latex immunonephelometric (Nephelometer analyzer, BNII, Siemens). Uromodulin (UM) was measured from urinary samples stored at -60°C using an enzyme-linked immunosorbent assay (Bio-Vendor, Cobas Roche) and standardized to urinary creatinine [indexed UM (iUM)]. The glomerular filtration rate (eGFR) was estimated using the CKD-EPI creatinine equation: {eGFR, ml/min/1.73 m2=141 x min (SCr/κ, 1)α x max(SCr/κ, 1)-1.209 x 0.993Age x 1.018 [if female] x 1.159 [if Black]} (14). Insulin resistance and pancreatic beta cell function were assessed using the HOMA method, i.e., HOMA-IR (Homeostasis Model Assessment for Insulin Resistance): [HOMA-IR = fasting plasma insulin x fasting plasma glucose)/22.5] and HOMA-β (Homeostasis Model Assessment of β-Cell Function) [HOMA ß (%ß) = HOMA-IR – (20 x fasting plasma insulin) / (fasting plasma glucose – 3.5)] (15). Transthoracic echocardiograms were performed in 143 participants by a single experienced operator. Left ventricular mass (LVM) was calculated and indexed in accordance with established echocardiographic methods (16).

Cardiovascular Risk Stratification Cardiovascular risk was assessed using the Framingham 10-Year Risk Score for General Cardiovascular Disease (FRS). Participants were categorized into low-risk (<5%) and elevated-risk (≥5%) groups (17).

Statistical Analysis Descriptive statistics were used to summarize the data. Continuous variables were reported as mean ± standard deviation (SD) or median with interquartile range (IQR), depending on normality. Categorical data were expressed as absolute and relative frequencies. Group differences in categorical variables were assessed using Chi-square or Fisher's exact tests. Continuous variables between two groups were analyzed using the Mann–Whitney U test; comparisons across three or more groups employed ANOVA (with Bonferroni or Scheffé post hoc tests) or the Kruskal–Wallis test (with Conover post hoc analysis). Correlations between numeric variables were examined using Spearman's rank correlation coefficient (ρ). Stepwise multivariate linear regression and logistic regression were applied to identify independent associations of interest. All statistical tests were two-tailed, with significance set at $\alpha = 0.05$. Analyses were conducted using MedCalc Statistical Software version 18.2.1 (MedCalc Software bvba, Ostend, Belgium; www.medcalc.org; 2018) and IBM SPSS Statistics version 23.0 (IBM Corp., Armonk, NY, USA).

Results

Baseline clinical and laboratory characteristics are summarized in table 1. Although not statistically significant, NT and PHT participants were slightly younger than those in the HT group. Gender distribution varied significantly ($p<0.001$). The PHT group had intermediate BMI ($p=0.009$) and waist circumference ($p=0.001$) values between NT and HT participants. Significant differences were observed in serum uric acid and fasting blood glucose, ($p<0.001$), HbA1c ($p=0.01$), insulin ($p=0.002$), HOMA-IR ($p=0.001$), total cholesterol ($p=0.04$), and triglycerides ($p<0.001$) levels across the three BP groups. However, glucose and HbA1c did not differ significantly between NT and PHT groups, while triglycerides and uric acid levels were elevated in both PHT and HT groups compared to NT. Kidney function markers (eGFR, ACR, and tubular markers), showed no significant differences. Sodium/potassium ratio and estimated salt intake were above recommended levels but did not differ across groups. A non-significant positive trend of increased urinary uromodulin with rising BP was observed.

Table 1. Baseline data of participants according to the JNC-7 blood pressure categories

Parameter	NT (n=103)	PHT (n=140)	HT (n=80)	P
SBP (mmHg)	108.3 (7.1)	126.9 (80.8)	143.9 (94.2)	<0.001*, †
DBP (mmHg)	70.9 (6.2)	80.8 (6.5)	94.2 (8.2)	<0.001*, †
Sex, m, [n (%)]	74 (71.8)	61 (42.7)	30 (37.5)	<0.001‡
Age (years)	36 (30–42)	37 (29–44)	39 (33–45)	0.07
BMI (kg/m²)	25.6 (22.5–28.7)	26.6 (23.8–30)	28.6 (24.7–32.6)	0.009§
WC (cm)	90 (79–100)	92 (84–102)	99.5 (89–110)	0.001§
Uric acid (µmol/L)	252 (211–307)	297 (244.5–370)	328 (274–412.8)	<0.001†
Glucose (mmol/L)	4.9 (4.5–5.2)	5.0 (4.6–5.3)	5.3 (4.8–5.6)	<0.001§
HbA1c (%)	5.1 (4.9–5.4)	5.1 (4.9–5.1)	5.2 (5.0–5.4)	0.01‖
Insulin (mIU/L)	9.3 (6.6–14.6)	13.4 (8.6–17.7)	14.9 (10–25.9)	0.002
HOMA-IR	1.9 (1.4–2.7)	2.9 (1.7–4.1)	3.5 (2.1–5.6)	0.001¶
Total cholesterol (mmol/L)	4.9 (4.3–5.6)	5.2 (4.6–5.7)	5.2 (4.6–5.9)	0.04†
HDL-cholesterol (mmol/L)	1.3 (1.1–1.6)	1.3 (1.1–1.7)	1.3 (1.0–1.6)	0.35
LDL-cholesterol (mmol/L)	3.0 (2.4–3.6)	3.1 (2.6–3.7)	3.1 (2.6–3.9)	0.32
Triglycerides (mmol/L)	0.9 (0.7–1.4)	1.0 (0.7–1.5)	1.4 (0.9–2.0)	<0.001†
Serum potassium (mmol/L)	4.3 (4.2–4.63)	4.4 (4.1–4.7)	4.3 (4.1–4.5)	0.09
Serum sodium (mmol/L)	140 (139–141)	140 (139–142)	140 (139–141)	0.48
Serum creatinine (µmol/L)	68 (63–76)	75 (65–86)	74 (65–86.3)	0.001¶
CKD-EPI eGFR (ml/min/1.73 m²)	107 (96.3–115)	105 (91–113)	104.5 (94–110)	0.32
ACR urine (mg/g creatinine)	4.14 (2.64–6.05)	4.1 (2.3–6.6)	4.7 (2.8–8.3)	0.54
A1mCR urine (mg/g creatinine)	5.6 (3.3–7.7)	4.3 (3.2–6.7)	5.1 (3.6–8.4)	0.07
Na/K ratio, urine	3.3 (2.5–4.5)	3.3 (2.4–5.2)	3.1 (2.4–4.1)	0.71
Uromodulin (mg/g creatinine)	43 (27–66)	42.9 (25.5–65.3)	40.6 (24.1–60.4)	0.73
C-reactive protein (mg/L)	1.8 (0.9–2.8)	1.2 (0.6–2.4)	2.0 (0.9–4.1)	0.05

Statistical test used for P values: Kruskal–Wallis test [median (25%-75%)]; * ANOVA; ‡ χ^2 test; post hoc Conover, as appropriate.
† Significant differences between NT vs. PHT, NT vs. HT, and PHT vs. HT
§ Significant differences between NT vs. HT and PHT vs. HT
‖ Significant differences between PHT vs. HT
¶ Significant differences between NT vs. PHT and NT vs. HT

Abbreviations:
NT – normotensive; PHT – prehypertensive; HT – hypertensive.
SBP – systolic blood pressure; DBP – diastolic blood pressure.
BMI – body mass index; WC – waist circumference.
HbA1c – glycated hemoglobin A1c; HOMA-IR – Homeostasis Model Assessment for Insulin Resistance.
HDL – high-density lipoprotein; LDL – low-density lipoprotein.
eGFR – estimated glomerular filtration rate; CKD-EPI – Chronic Kidney Disease Epidemiology Collaboration.
ACR – albumin/creatinine ratio in spot urine.
A1mCR – alpha-1-microglobulin/creatinine ratio in spot urine.
Na/K ratio – sodium/potassium ratio in spot urine.

Table 2. Echocardiographic data of participants according to the JNC-7 BP categories.

Parameter	NT (n=41)	PHT (n=52)	HT (n=50)	P
LA (mm)	32.5 (30.3 - 36)	33 (30 - 36)	36.5 (32.8 - 39)	0.007*
LAVI (ml/m2)	20.8 (16.8 – 23.6)	19.1 (16.5 – 22.7)	21.2 (17.4 - 26)	0.25
LV (mm)	47 (44 – 50.5)	49 (45 - 51)	46.5 (43 - 52)	0.43
IVS (mm)	10 (8 - 10)	10 (9 - 11)	10 (9 - 12)	0.02†
LVPW (mm)	9 (8 – 10.5)	10 (9 - 11)	11 (10 - 12)	<0.001‡
LVM (g)	142.1 (122.3 – 180.9)	163.72 (146.4 -193.8)	190.4 (145.9 – 228.3)	0.001‡
LVMI (g/m2)	77.5 (67.6-88.3)	84.3 (73.3-95.9)	87.7 (74.3-107.4)	0.01†
RWT (cm)	0.4 (0.4 – 0.4)	0.4 (0.4-0.5)	0.5 (0.4 – 0.6)	<0.00*
E/A	1.3 (1 – 1.5)	1.3 (1 – 1.5)	1.3 (1 – 1.5)	0.89
E/e′	7 (5.6 – 8.7)	7.4 (6.3 - 9)	8.2 (6.8 – 9.4)	0.03†
SV (ml)	67.5 (58.5 – 82.8)	74 (63.5 - 86)	74 (61 – 95.5)	0.24
EFLV (%)	66 (62 - 70)	64 (60 - 69)	67 (62 – 68.3)	0.34

Statistical test used for the P value: Kruskal–Wallis, [median (25%-75%) and interquartile range]; post hoc Conover, as appropriate.
*Significant differences between NT vs.HT and PHT vs.HT
†Significant differences between NT vs.HT
‡Significant differences between NT vs. PHT, NT vs.HT, and PHT vs.HT

Abbreviations:
NT, normotensive; PHT, prehypertensive; HT, hypertensive.
LA, left atrium; LAVI, indexed left atrium volume; LV, left ventricle.
IVS, interventricular septum; LVPW, left ventricular posterior wall; LVM, left ventricular mass.
LVMI, left ventricular mass indexed; RWT, relative wall thickness.
E/A, the ratio of peak velocity blood flow from left ventricular relaxation in early diastole (the E wave) to peak velocity flow in late diastole caused by atrial contraction (the A wave).
E/e', the ratio of early filling velocity on transmittal Doppler (E) with the early relaxation velocity on tissue Doppler (E′).
SV, stroke volume; EFLV, left ventricular ejection fraction.

Echocardiographic data (table 2) revealed progressive increases in IVS, LVPW, LVM, RWT, LVMI, and E/e′ ratio across BP categories (all $p<0.05$). Notably, PHT participants exhibited early signs of cardiac remodeling without significant differences from HT individuals in several parameters. Framingham risk scoring showed a stepwise increase in the proportion of high-risk individuals across BP groups (7.9% NT, 24.6% PHT, 48.1% HT; $p<0.05$). In multivariate logistic regression, triglycerides emerged as the only independent predictor of elevated CV riskin the PHT group ($Exp(\beta) = 1.88$, 95% CI: 1.08–3.28; $p = 0.03$). ROC analysis yielded an AUC of 0.706 for triglycerides, with a cutoff of >1.3 mmol/L indicating elevated CV risk.

Table 3. Final model of significant predictors of CVR in the PHT group.

Parameter	ß	Standard error	Wald	p	Odds ratio (Exp ß)	95% CI za Exp ß
Triglycerides (mmol/L)	0.63	0.28	4.96	**0.03**	1.88	1.08 do 3.28
Constant	-2.8	0.73	15.0	**<0.001**		

ß, regression coefficient; CI, confidence interval

Table 4. ROC curve parameters for CVR in the PHT group.

Parameters	AUC	95% CI	Sensitivity	Specificity	Cut-off	Youden index	P
Triglycerides (mmol/L)	0.706	0.622-0.781	58.8	74.8	> 1.3	0.34	**<0.001**

AUC, area under the curve

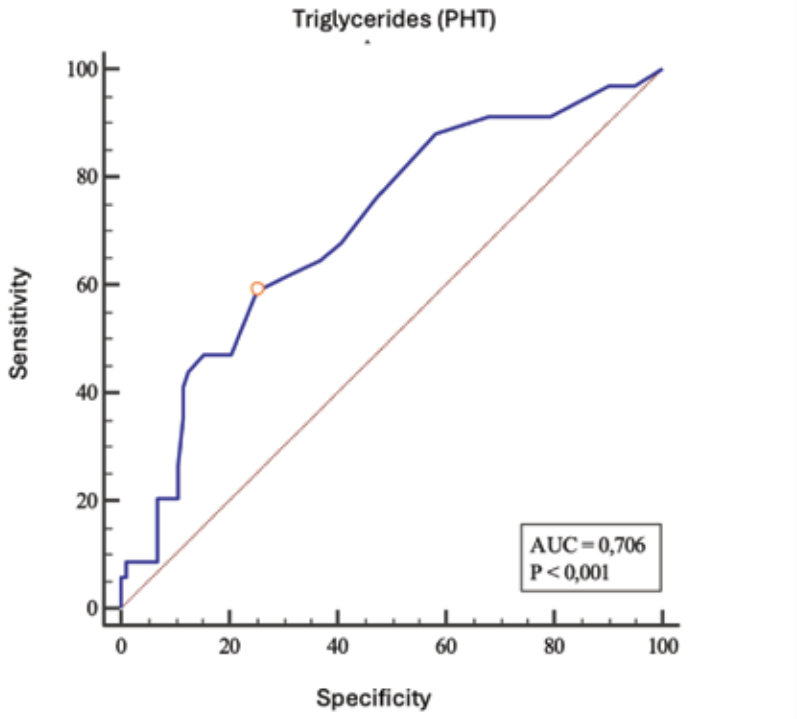

Figure 1. ROC analysis of sensitivity, specificity, and cut-off value for triglycerides regarding CVR in the PHT group.

Discussion

Our findings suggest that hypertriglyceridemia is an early and significant metabolic abnormality in young, untreated individuals with PHT. Compared to NT and HT participants, the PHT group demonstrated intermediate but distinctly adverse cardiometabolic profiles. Elevated triglyceride levels, alongside higher HOMA-IR and serum uric acid, were independently associated with increased CV risk even in this low-risk, young adult population. This is a finding that adds to growing evidence but remains underrepresented in the literature (12,18). These observations align with prior studies. Grotto et al. and Chiang et al. found that individuals with PHT exhibited higher levels of fasting blood glucose, triglycerides, and BMI compared to NT group (12,18). Although the HT group in our study showed more severe metabolic derangements, the metabolic shift begins early, supporting the rationale for early detection and targeted prevention strategies. We also found evidence of early cardiac remodeling in the PHT group, including increased left ventricular mass and diastolic dysfunction, while kidney function markers remained similar across BP categories. This suggests that early hypertension-mediated organ damage manifest initially in the heart rather than the kidneys. Previous studies have shown similar cardiac alterations in PHT, such as early changes in left atrial mechanics and geometry, and an association between left ventricular hypertrophy and HT progression (19–21). In contrast, although albuminuria and GFR have been reported in other PHT populations, especially in older cohorts or those with clustered risk factors, we did not observe renal impairment in our younger, healthier participants (22). A stepwise increase in Framingham Risk Score (FRS) was evident across BP groups, with PHT individuals already showing a markedly higher proportion of elevated CV risk. Among all evaluated variables, triglycerides emerged as an independent predictor of elevated FRS in the PHT group. This supports existing genetic, epidemiological, and clinical research indicating that triglyceride-rich lipoproteins (TRLs) and their components, including ApoC3 and ApoE, contribute causally to atherosclerotic CV disease (23-26). Moreover, Mendelian randomization studies and real-world data have confirmed the association between elevated TG levels and increased CV events and all-cause mortality, even in populations with optimal LDL-cholesterol levels (27-31).

Our study has several strengths, including a relatively homogeneous and untreated cohort of young adults with preserved kiney function, and the use of standardized measurement protocols. Nonetheless, it is limited by its cross-sectional design single-center study with unbalanced gender distribution, and reliance on fasting triglyceride levels — despite evidence that nonfasting trigylcerides may better reflect real-world risk (27,29). Additionally, we captured triglyceride levels at a single time point, potentially missing postprandial triglyceride elevations in some individuals.

In conclusion, hypertriglyceridemia should be recognized as an early and independent marker of CV risk in young individuals with PHT. Incorporating triglyceride measurement into routine CV risk assessment may enable timely interventions to prevent the progression to HT and CV disease in this under-recognized group.

Preprint Disclosure:

A version of this manuscript was previously posted on a preprint server, DOI: [insert preprint DOI].

Funding:

This research was supported by the scientific research project *"Endemic Nephropathy in Croatia – Epidemiology, Diagnostics, and Etiopathogenesis"* (project code: 108-0000000-0329), funded by the Ministry of Health of the Republic of Croatia, and by the research grant of the Croatian Hypertension League. The research did not receive a specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest:

The authors declare no conflicts of interest.

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Quality Management as a Tool for Improving the Performance of Healthcare Organizations at Different Levels

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This article was submitted to RAD CASA - Medical Sciences as the original article

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 11 February 2025
Accepted: 29 May 2025
Published: 25 June 2025

Citation:
Medjedovic E, Šehić–Kršlak S, Begić E, Čarovac A, Suljević A, Salibašić M. Quality Management as a Tool for Improving the Performance of Healthcare Organizations at Different Levels
567=70-71 (2025): 28-34
DOI: 10.21857/ydkx2cvoq9

Abstract

Introduction: Healthcare organizations are faced with numerous problems, including a chronic lack of financial resources.

Aim: To determine the difference in familiarity with the standards related to the quality management system between the primary, secondary and tertiary levels of health care, to determine the difference in the implementation of the quality system; determine the connection between the implementation of the quality system and the satisfaction of service users – patient.

Materials and methods: Statistical processing of all data from the survey questionnaire was performed in the SPSS 17.0 package (SPSS Inc.). The level of statistical significance was set at 0.05, and all confidence intervals are given at the 95% level. The normality of the distributions was checked by Shapiro-Wilk tests when the samples were smaller than n=30, and by the Kolmogorov-Smirnov test for larger samples. Given that in most cases they indicated statistically significant deviations from normal distributions, the median and interquartile ranges were used as measures of central tendency and dispersion. Mutual comparisons of all three levels of health activity were analyzed with the Mann-Whitney U test, with Bonferroni's correction of the level of statistical significance. The survey questionnaire was sent to the addresses of a total of 110 BiH health institutions at the primary, secondary and tertiary levels of health care. In the period June/July 2024, a total of 86 completed survey questionnaires were collected, which represents 76,18 % of all respondents.

Conclusion: Research on a sample of primary, secondary and tertiary healthcare institutions in Bosnia and Herzegovina showed that the knowledge and application of the quality management system is still insufficient and points to the need to raise awareness of the importance of the application of the quality management system. Clinical hospital centers, as expected, given the application of the highest standard of medicine and the highest level of provision of health services, showed: greater knowledge of the importance of the quality management system and representation of quality assurance requirements of the ISO standard. The research also found that healthcare institutions that use international standards of quality management systems perform systematic monitoring of patient satisfaction with services provided statistically significantly more often ($\chi 2$ = 5.134; ss = 1; P = 0.032; contingency coef-

ficient = 0.237). 47.1% of them carried out systematic monitoring of patients, compared to 20.3% of institutions that did not implement international norms of the quality management system.

Keywords: quality management, healthcare organizations, service users

Sažetak

Upravljanje kvalitetom kao alat za poboljšanje učinkovitosti zdravstvenih organizacija na različitim razinama

Uvod: Zdravstvene organizacije suočavaju se s brojnim problemima, uključujući kronični nedostatak financijskih sredstava.

Cilj: Utvrditi razliku u poznavanju standarda vezanih uz sustav upravljanja kvalitetom između primarne, sekundarne i tercijarne razine zdravstvene zaštite, utvrditi razliku u implementaciji sustava kvalitete; utvrditi povezanost između implementacije sustava kvalitete i zadovoljstva korisnika usluga – pacijenta.

Materijali i metode: Statistička obrada svih podataka iz anketnog upitnika provedena je u SPSS 17.0 paketu (SPSS Inc.). Razina statističke značajnosti postavljena je na 0,05, a svi intervali pouzdanosti dati su na razini od 95%. Normalnost distribucija provjerena je Shapiro-Wilk testovima kada su uzorci bili manji od n=30, a Kolmogorov-Smirnov testom za veće uzorke. S obzirom na to da su u većini slučajeva ukazivale na statistički značajna odstupanja od normalnih distribucija, kao mjere centralne tendencije i disperzije korišteni su medijan i interkvartilni rasponi. Međusobne usporedbe sve tri razine zdravstvene aktivnosti analizirane su Mann-Whitneyjevim U testom, s Bonferronijevom korekcijom razine statističke značajnosti. Anketni upitnik poslan je na adrese ukupno 110 zdravstvenih ustanova u BiH na primarnoj, sekundarnoj i tercijarnoj razini zdravstvene zaštite. U razdoblju lipanj/srpanj 2024. prikupljeno je ukupno 86 ispunjenih anketnih upitnika, što predstavlja 76,18 % svih ispitanika.

Rasprava: Istraživanje na uzorku zdravstvenih ustanova primarne, sekundarne i tercijarne zdravstvene zaštite u Bosni i Hercegovini pokazalo je da je poznavanje i primjena sustava upravljanja kvalitetom još uvijek nedovoljno te ukazuje na potrebu podizanja svijesti o važnosti primjene sustava upravljanja kvalitetom. Klinički bolnički centri, očekivano, s obzirom na primjenu najvišeg standarda medicine i najvišu razinu pružanja zdravstvenih usluga, pokazali su: veće poznavanje važnosti sustava upravljanja kvalitetom i zastupljenost zahtjeva osiguranja kvalitete ISO standarda. Istraživanje je također utvrdilo da zdravstvene ustanove koje koriste međunarodne standarde sustava upravljanja kvalitetom statistički značajno češće provode sustavno praćenje zadovoljstva pacijenata pruženim uslugama ($\chi 2$ = 5,134; ss = 1; P = 0,032; koeficijent kontingencije = 0,237). Njih 47,1% provodilo je sustavno praćenje pacijenata, u usporedbi s 20,3% ustanova koje nisu implementirale međunarodne norme sustava upravljanja kvalitetom.

Ključne riječi: upravljanje kvalitetom, zdravstvene organizacije, korisnici usluga

Introduction

A good healthcare system provides patients with greater security, which contributes to preserving working ability and increasing productivity [1]. Quality improvements are primarily focused on improving quality of care and other patient service outcomes [2]. Kodate [3] analyzed the national health systems of England, Sweden and Japan and concluded that government requirements, created in response to the needs and expectations of the public, represent the basis for understanding changes in health policy. Furthermore, changes in health policy are influenced by dynamic relations between the public and governing structures. The procedure for establishing the Quality Management System in the organizational system is defined by ten steps shown in the picture. The first and most important requirement is that it should be the decision of the top management, which is expressed by the realization of the basic task of defining the organization's policy based on the vision and, within it, the quality policy" (standard ISO 9001). Literary data indicate that the establishment of a quality management system in a healthcare institution is defined through:

- evidence-based health care quality improvement;
- patient and healthcare provider safety;
- verification user satisfaction and service provider;
- transparency of work;
- responsible management with rational use of infrastructure, human resources and modern technologies;
- organization continuity of care, etc.

International standard ISO/IWA 1 - *International Workshop Agreement - Quality Management Systems* - Guidelines for Process Improvements in Health Service Organizations provides guidelines for the management of any type of organizational system which deals with the provision of health services. It is fully compliant with ISO 9001 and ISO 9004, and the guidelines have been expanded to take into account the specificity and special requirements of the health service.

Countries around the world are mostly facing the problem of high increase in health care costs. Increasing costs are a reflection of rising technology costs, an aging population, an increase in the number of chronically ill patients, and an increase in the demand for healthcare. An additional difficulty is determining the true value of healthcare services [4].

The most frequently used tool for measuring the quality of the provided service from the perspective of the user is the *SERVQUAL* instrument. The instrument is based on measuring the gap between users' perceptions and expectations with regard to different elements of service quality. It consists of five dimensions. These are: tangible elements, reliability, responsibility, safety, empathy [5]. Implementation of modern models at the level of tertiary health care is particularly popular [6].

Research Results

The analysis of the application of the quality management system in the Bosnian-Herzegovinian health care system was carried out through a survey using a specially structured survey questionnaire. The target population of the research was managers of health institutions with three levels of health activity.

In relation to the assessment of the state of the quality system in the health care system, it was investigated to what extent international standards for building a quality management system (ISO 9001:2008) or some other quality improvement model are used in health care institutions and to what extent their application affects the level of improvement compared to several dimensions of quality (clinical effectiveness, safety, efficiency, management, patient orientation and staff status), which are the anticipated advantages that the management of BH. health institutions expect the introduction of a quality management system, i.e. what are the anticipated obstacles for its application in the business of health institutions. The research established the fact that 80% (69/86) of healthcare institutions do not apply international standards for building a quality system (ISO 9001:2008). Only 23% (20/86) of the analyzed health institutions apply international norms for the construction of quality management systems.

If we compare the results with the research of health institutions abroad, it is evident that the health care of Bosnia and Herzegovina lags behind significantly. Of the 254 hospitals in South Korea, 33.9% of them have implemented the ISO 9001 norm, and even 60.6% of them apply full quality management [7].

Of the surveyed healthcare institutions that stated that they use the international norm for building a quality system, only 8% of them use it in the entire institution or in most organizational units, and 19% of them in a few or only one organizational unit. It can be concluded that the management of healthcare institutions does not recognize the importance of applying international quality system standards. The attitude of health management in relation to the advantages brought by the introduction of the quality system is reflected in the following statements: the most common are the reduction of costs, a higher level of service and patient satisfaction (more than 60%), while in relation to transparency, the advantages of the introduction of the quality system are seen by only 18.4 % of respondents.

However, in relation to the benefits that the introduction of the quality system brings to the staff of the health institution, i.e. to the quality of the employees' performance, only 9.1% of them state an increase in staff satisfaction, and 80.1% of respondents do not think that the introduction of the quality system will increase the staff's responsibilities towards patients. Optimism was not expressed or in the assessment of the advantages of the quality system in relation to better control of internal processes, uniformity and systematization of business processes and organization, namely, more than 70% of respondents do not see the advantages of introducing a quality management system for the operation of the institution and the management of the provision of health services. As the greatest potential obstacle for the establishment of a quality management system, the managers in the same proportion (63.5%) pointed out the lack of financial resources considering the high costs of introducing the system and insufficient human resources for the implementation of the quality management system.

In 55% of the submitted answers, insufficient knowledge of the functioning mechanisms of the quality management system is stated as the reason for not applying the quality management system, while the length and complexity of the system introduction procedure is shown as the reason for not applying the quality management system in 25.1% of the received answers.

According to the obtained results, it can be concluded that the establishment and application of the quality management system in BH healthcare institutions is insufficiently developed, which is supported by the fact that only 28% of healthcare institutions have completed the certification of the quality management system. The conducted research attempted to examine how much awareness of the importance of quality management and familiarity with the standards related to the quality management system differ between the primary, secondary and tertiary levels of health care. An effort was made to determine at which level of health care the quality management system is mostly applied.

In relation to the familiarity with the standards related to the quality management system, a significant difference was determined in the assessment of familiarity with the mechanisms with regard to the three levels of health activity. The average assessment of knowledge of mechanisms was the lowest at the primary level of health care, and the participants rated their knowledge

as superficial, while the participants of secondary and tertiary health care assessed their knowledge of the functioning mechanisms of the quality management system as good. A statistically significant difference was found between primary and secondary types of health care (Mann - Whitney U = 379.5; Z = - 2.927; P = 0.003). The implementation of the quality management system of the health sector is best acquainted with the functioning mechanisms of the quality management system, because at that level the competences of employees are the greatest, as well as the complexity of the activities and processes that take place within the tertiary level.

Table 1: Application of standards in healthcare organizations

Application of standards in health organizations	66%
Internal protocols	54%
Total quality management (TQM)	63%
Periodic patient satisfaction survey	38%
Patterns	56%
Laws and regulations	52,3%
Others	-

Source: Author's research

Table 2: Advantages of introducing quality management standards in healthcare institutions

A higher level of service	43,2%
Greater staff satisfaction	51,8%
Greater hospitality of patients	47,5%
Transparency	52,3%
Greater responsibility of staff towards patients	34,5%
Reducing the risk of medical error	69,2%
Reduce costs	53%
Better control of internal costs	51,7%

Source: Author's research

In relation to the familiarity with the standards related to the quality management system, a significant difference was found in the assessment of the familiarity with the mechanisms with regard to the three levels of healthcare activity. The average assessment of knowledge of mechanisms was the lowest at the primary level of health care, and the participants rated their knowledge as superficial, while the participants of secondary and tertiary health care assessed their knowledge of the functioning mechanisms of the quality management system as good. A statistically significant difference was found between the primary and secondary types of health activity (Mann - Whitney U = 379.5; Z = - 2.927; P = 0.003), while primary and tertiary, as well as secondary and tertiary types of activity did not differ statistically significantly from each other. It is expected that managers at the tertiary level of health care are best acquainted with the functioning mechanisms of the quality management system, because at that level the competencies of the employees are the greatest, as well as the complexity of the activities and processes that take place within the tertiary level.

Table 3 Level of health activity

Level of health activity	N	K-S/S-WP	Median	IQR	P-effect
Primary	33	<0,001	¨2	2-3	0,007-0.89
Secondary	37	<0,001	3	3-3	
Tertiary	16	0,019	3	2,3-3,8	

Source: Author's research

KS/SW P = Kolmogorov-Smirnov test of normality of distribution for samples greater than 30 or Shapiro - Wilk test of normality of distribution for samples less than 30, level of statistical significance; IQR = interquartile range; P = Kruskal - Wallis test difference between more than two groups on a continuous variable; the level of statistical significance, that is, the probability of type i error (alpha); effect = standardized effect size measure of the day for statistically significant results, η2 for Kruskal - Wallis test.

Discussion

Regarding the dimensions of health service quality, previous research has shown that reliability, empathy and tangible elements have the greatest importance [8]. Kumaraswamy [9] concluded that the factors of quality of highly educated service are: physician's behavior, friendly staff, environment and work performance. The most common customer complaints include: long waiting lists, high costs, unfriendly and indifferent staff [10]. Therefore, it is important to identify the parameters of the quality of the health service with regard to the usefulness for the organization, patients and society. Other authors point out that patients cannot distinguish the care of staff (functional performance) from the treatment procedure (technical performance) of healthcare providers [11]. That is why many patients evaluate the quality of service based on functional aspects of technical performance. Furthermore, they state that non-technical interventions influence the evaluation of the overall quality of the health service and that these aspects are more important than technical ones. Therefore, the quality of the health service should not be evaluated solely on the basis of user ratings, i.e. patients. Other approaches to measuring the quality of healthcare services most often include monitoring certain quantitative indicators or examining employee perceptions in order to examine the extent to which certain practices, tools and models are applied in healthcare institutions. Lee [7,12] concluded that quality control (61.8%) and total quality management (60.6%) are most often applied within the healthcare system. The bh. health care system is based on the principles of social insurance and according to the applied health care model, it is closest to the Bismarck financing model. In contrast to some other systems where tax is the basic financing instrument, this is a model of mandatory social health insurance based on a contribution from wages.

Quality and constant quality improvement are a priority of the national health policy at all levels of health care and are the result of measures taken in accordance with modern needs regarding health procedures that ensure the highest possible favorable outcome of individual diagnosis and treatment procedures. There are two sets of standards in Bosnia and Herzegovina: mandatory quality standards and the manner of their application, and accreditation standards in accordance with which the accreditation procedure for hospital healthcare institutions is carried out. The quality policy should implement a system of continuous improvement of the quality of health care and patient safety in accordance with EU directives and recommendations of the Council of Europe, the establishment of accepted quality standards, a national program of quality indicators (indicators), a risk management system, internal and external quality assessment, strengthening the role of the patient and financing of the quality system with recognition and reward for improvement.

Cronin and Taylor criticized the use of gap analysis results in the measuring service quality and concluded that service quality is measured only by the perception of service users sufficient. There are also numerous disagreements as to whether the scale for quality measurement universally apply services between different service activities and whether there is any connection at all between the satisfaction of service users and quality.

This research once again confirmed that the concept of quality of health services is multidimensional and that it is with the application of the SERVQUAL model, it is possible to find out the expectations, needs and wishes of the users of health institutions in a simple and effective way, as well as their attitudes about the services received, and then use them for the purpose of creating a quality offer. The results of this research confirmed that the concept of health services is multidimensional. The needs, requirements and expectations of the users are constantly changing, so it is necessary to carry out continuous measurements in order to know what the users expect and whether the provided service satisfies their needs in every segment needs and expectations.

Conclusion

The research confirmed the differences in the understanding and application of the quality management system with regard to different levels of health care. Managers at the primary level of healthcare are the least familiar with and apply quality management systems, while the situation is somewhat better at the secondary and tertiary levels. Regarding the benefits of introducing a quality system for the operation of the institution and the impact on the satisfaction and increased responsibility of health care workers, about 80% of the respondents were of the opinion that there is no significant benefit from it, which can lead to the conclusion that they consider the current quality of health service performance to be satisfactory

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Behavioral Patterns and Their Contribution to Cardio-Kidney-Metabolic Health in the Student Population

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Abstract

This study aims to examine behavioral patterns among university students, including dietary habits, physical activity, sedentary behavior, alcohol consumption and their association with cardio-kidney-metabolic (CKM) health and risk. A cross-sectional survey was conducted among 1,490 students in Croatia. The results indicate that a substantial proportion of students engage in health-risk behaviors, such as insufficient physical activity, prolonged sedentary time, and unhealthy eating patterns. Self-reported data revealed the presence of hypertension, dyslipidemia, type 1 diabetes, chronic kidney disease, and overweight within the student population. Gender-specific associations were observed: lower physical activity was linked to hypertension in males and to elevated lipid levels and overweight in females. Furthermore, poor dietary habits, smoking, and alcohol use were significantly associated with certain health outcomes. These findings highlight the importance of early identification and intervention targeting risky behaviors in student populations to prevent future CKM complications.

Keywords: behavioral patterns, cardio-kidney-metabolic health, physical activity, sedentary behavior, dietary habits, alcohol consumption, student health, risk factors

Sažetak:

Obrasci ponašanja i njihov doprinos kardio-bubrežno-metaboličkom zdravlju u studentskoj populaciji

Ovo istraživanje imalo je za cilj ispitati obrasce ponašanja među studentskom populacijom, uključujući prehrambene navike, tjelesnu aktivnost, sjedilačko ponašanje te konzumaciju alkohola i duhana, i njihovu povezanost s kardiovaskularnim, bubrežnim i metaboličkim (CKM) zdravljem i rizicima. Provedeno je presječno istraživanje na uzorku od 1.490 studenata iz različitih sveučilišta u Hrvatskoj. Rezultati pokazuju da značajan udio studenata prakticira rizična ponašanja po zdravlje, poput nedovoljne tjelesne aktivnosti, produženog sjedenja (više od 7 sati dnevno), te učestale konzumacije brze hrane, prerađenog mesa, zaslađenih napitaka i energetskih pića. Samoprocjenom su studenti naveli prisutnost zdravstvenih tegoba kao što su hipertenzija, dislipidemija, dijabetes tipa 1, kronična bubrežna bolest te prekomjerna tjelesna masa. Uočene su razlike prema spolu: kod muških studenata niža razina tjelesne aktivnosti značajno je povezana s hipertenzijom, dok je nezdrava prehrana povezana s dijabetesom i bubrežnim smetnjama. Kod studentica, niska tjelesna aktivnost povezana je s povišenim masnoćama i prekomjernom tjelesnom masom, a pretjeran unos kuhinjske soli i prerađene hrane s višim arterijskim tlakom. Pušenje i konzumacija alkohola također su pokazali značajnu povezanost s hipertenzijom i debljinom kod žena. Dobiveni nalazi naglašavaju potrebu za spolno specifičnim preventivnim strategijama i ranim intervencijama usmjerenima na promicanje zdravih životnih navika u studentskoj populaciji kako bi se smanjili dugoročni kardio reno metabolički rizici.

Ključne riječi: obrasci ponašanja, kardio-reno-metabolički rizik, tjelesna aktivnost, sjedilačko ponašanje, prehrambene navike, konzumacija alkohola, zdravlje studenata, rizični faktori

OPEN ACCESS

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This article was submitted to RAD CASA - Medical Sciences as the original article

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 8 May 2025
Accepted: 29 May 2025
Published: 25 June 2025

Citation:
Ćurković S, Jelaković B, Jovanović Ž. Behavioral Patterns and Their Contribution to Cardio-Kidney-Metabolic Health in the Student Population

567=70-71 (2025): 36-42
DOI: 10.21857/m16wjcwnv9

Introduction

Cardio-kidney-metabolic (CKM) syndrome, characterized by a cluster of interrelated risk factors including hypertension, abdominal obesity, dyslipidemia, and insulin resistance, significantly increases the risk of developing cardiovascular disease, type 2 diabetes, and chronic kidney disease (Cases et al., 2023). In recent years, there has been a growing emphasis on the early detection of risky health behaviors, especially among young adults, as a means to prevent the onset of chronic diseases later in life (Yandutkina at al., 2024). University students represent a particularly vulnerable group undergoing substantial lifestyle transitions, such as decreased physical activity, increased sedentary behavior, and irregular eating habits—all of which can negatively impact metabolism and contribute to early manifestations of CRM risk (Topbaş at al. 2022). Previous studies have consistently reported that students tend to consume insufficient amounts of fruits and vegetables while frequently consuming fast food and sugar-sweetened beverages (Almoraie et al. 2024). Additionally, the high prevalence of smoking and alcohol consumption among university students further contributes to their overall metabolic and cardiovascular risk (Shin et al. 2022). Although regular physical activity and a balanced diet are recognized as essential protective factors against CRM syndrome, recent findings from both European and Croatian contexts reveal alarmingly low levels of physical activity among students (Strain at al. 2024). Furthermore, sedentary behavior has emerged as an independent risk factor for CKM health, regardless of total physical activity levels (Henshel et al. 2022., WHO, 2022). Recent data also suggest that screen time, irregular sleep patterns, and chronic stress may play a compounding role in elevating CKM risk among young adults (Richardson at al. 2025). Despite increasing awareness of healthy lifestyle recommendations, many students struggle to adopt and maintain behaviors that support long-term metabolic and cardiovascular health.

The aim of this study is to analyze behavioral patterns among university students—including dietary habits, physical activity, sedentary behavior, alcohol consumption, and smoking—in order to identify risk factors that may indicate elevated CKM risk.

Hypotheses

H1: Lower levels of physical activity are associated with a higher prevalence of diagnosed hypertension, dyslipidemia, and overweight in the student population, indicating increased CRM risk.

H2: Unhealthy dietary habits (e.g., infrequent consumption of fruits and vegetables, frequent intake of fast food and sugar-sweetened beverages) are associated with poorer self-rated health and the presence of CKM risk markers among students.

Methods

Sample

The study included 1,490 undergraduate students from various universities across the Republic of Croatia. A convenience sampling method was employed, with voluntary and anonymous participation. Only adult students (aged 18 and above) who completed the entire questionnaire were included in the analysis. The data collection took place during the summer semester of the 2024/2025 academic year.

Instrument

Data were collected using a structured questionnaire comprising several thematic sections:

- Physical activity and sedentary behavior – questions related to engagement in physical activity for fitness purposes and the average daily duration of sedentary behavior.
- Dietary habits – frequency of fruit and vegetable consumption, use of added salt, and intake of fast food, carbonated beverages, and sugar-sweetened drinks.
- Harmful habits – alcohol consumption and smoking behavior.
- Diagnosed conditions and health status – self-reported presence of medical conditions including hypertension, elevated blood lipids, type 1 diabetes, kidney dysfunction, and overweight status, considered as indicators of potential cardio-renal-metabolic (CRM) risk.

The questionnaire was administered online using Google Forms. Participants were informed about the purpose of the study and the anonymity of their responses. The study adhered to ethical standards applicable within the academic community and was approved by the Ethics Committee of the Croatian Academic Sports Federation.

Data Analysis

Data were analyzed using the SAS statistical software package (version 26.0). Descriptive statistics were conducted, including frequencies, percentages, means, and standard deviations. Group differences were assessed using the chi-square test. After establishing a statistically significant association between the observed categorical variables using the chi-square test, Cramer's V was calculated to assess the strength of this association. The significance level was set at $p < 0.05$.

Results

A total of $N = 1,490$ students participated in the study, with 59.26% identifying as female. The mean age of participants was 21.46 years (SD = 3.66). The majority of the sample consisted of students aged 20–22 years (n = 653), followed by those aged 18–20 years (31.48%). All scientific fields were represented, with the largest proportion from technical sciences (27.99%), social sciences (26.58%), biomedical sciences (17.99%), and natural sciences (14.83%). Table 1 presents the prevalence of self-reported health-related behaviors among students, including both health-promoting behaviors (such as physical activity and consumption of fruits and vegetables) and health-risk behaviors (including prolonged sitting, excessive salt and processed meat intake, fast and processed food consumption, sugary and carbonated beverages, energy drinks), as well as alcohol use and smoking habits.

Table 1. Descriptive indicators of student health-related behaviors

No.	Self-assessed health-related behaviors of student	% total	M	F
1	**Physical activity (recommended level met)**	43,42%	53,38%	36,58%
2	**Sitting <7 hours per day**	38,39%	37,56%	38,96%
3	**Fruits and vegetables ≥ 4 times per week**	50,81%	47,45%	53,12%
4	**Added salt and processed meats ≥ 4 times per week**	32,82%	32,46%	23,07%
5	**Fast food ≥ 2 times per week**	54,43%	53,23%	65,17%
6	**Sugary and carbonated drinks ≥ 2 times per week**	49,99%	56,01%	45,87%
7	**Energy drinks ≥ 2 times per week**	14,29%	20,27%	10,19%
8	**Alcohol (frequent or daily consumption)**	14,70%	22,73%	9,17%
9	**Smoking (regular or daily)**	29,67%	26,69%	31,71%

The analysis shows that only 43.42% of students meet the recommended levels of physical activity, with men being significantly more active (53.38%) than women (36.58%). The majority of students (61.61%) sit for more than 7 hours a day, with no significant gender differences. Half of the students (50.81%) consume fruits and vegetables more than four times per week, with slightly healthier habits reported by women (53.12%). Unhealthy habits, such as frequent addition of salt and consumption of processed meats, were reported by 32.82% of students. Fast food is consumed more than twice a week by 54.43% of students, with women being more frequent consumers (65.17%).

Nearly half (49.99%) consume sugary beverages more than twice a week, more commonly among men (56.01%). Energy drinks are consumed by 14.29% of students, with a notable gender difference (20.27% of men vs. 10.19% of women). Regular alcohol consumption was reported by 14.70% of students, with men (22.73%) drinking significantly more frequently than women (9.17%). Smoking is present in 29.67% of students, with no major gender differences (26.69% of men and 31.71% of women). In addition, self-reported existing diagnoses or health conditions were examined. Descriptive indicators of the surveyed sample are presented in Table 2.

Table 2. Health Status of Students

DIAGNOSED CONDITIONS	TOTAL	M	F
Hypertension	8,66%	10,54%	7,36%
Elevated blood lipids	6,71%	4,45%	8,27%
Type 1 diabetes	3,96%	2,14%	5,21%
Chronic kidney disease	3,89%	2,31%	4,98%
Overweight	18,0%	16,3%	19,8%

In the overall sample of students, 8.66% reported a diagnosis of hypertension, 6.71% elevated blood lipids, 3.96% type 1 diabetes, 3.89% chronic kidney disease, and 18% overweight. When examining by gender, hypertension was more commonly reported among men, while elevated blood lipids, type 1 diabetes, kidney disease, and overweight were slightly more prevalent among women.

Association Between Lifestyle Habits and Diagnosed Conditions

The analysis revealed several significant associations between acquired lifestyle habits and reported health conditions, with differences by gender. Among male students, a statistically significant association was found between the level of physical activity and a diagnosis of hypertension (χ^2 = 11.98; p = 0.017; Cramer's V = 0.1405), with students who reported lower levels of physical activity more frequently diagnosed with hypertension. Among female students, significant associations were found with elevated blood lipids (χ^2 = 10.25, p = 0.036; Cramer's V = 0.1078) and overweight (χ^2 = 17.97, p = 0.001; Cramer's V = 0.1427). These results suggest that insufficient physical activity among female students may have a greater impact on metabolic risks (dyslipidemia and obesity) than on the early development of hypertension. The findings indicate that a lower level of physical activity may be associated with a higher risk of certain CKM conditions, especially among female students. Prolonged sitting for more than seven hours a day was not associated with most reported health conditions in students, except for a weak but significant association with chronic kidney disease in women (χ^2 = 13.95, p = 0.007), indicating that a sedentary lifestyle may be a risk factor for the development of kidney function impairment in the female population. The frequency of fruit and vegetable consumption showed a significant association with chronic kidney disease in women (χ^2 = 9.77, p = 0.044; Cramer's V = 0.1052), while the addition of extra salt to food and consumption of processed products were also significantly associated with hypertension in women (χ^2 = 11.5, p = 0.021; Cramer's V = 0.1142).

Table 3. Relationship Between Adopted Habits and Diagnosed Health Conditions

		Hypertension		Elevated blood lipids		Type 1 diabetes		Chronic Kidney disease		Overweight	
		X^2	p	X^2	p	X^2	p	X^2	p	X^2	p
Physical Activity	**M**	11.98	**0.017**	3.27	0.513	0.75	0.944	2.35	0.670	6.44	0.168
	F	6.37	0.173	10.25	**0.036**	4.76	0.312	5.82	0.212	17.97	**0.001**
Sitting	**M**	5.20	0.266	2.20	0.697	8.34	0.079	0.66	0.955	3.37	0.497
	F	1.17	0.117	7.09	0.130	5.25	0.262	13.95	0.007	2.73	0.600
Fruits and Vegetables / per week	**M**	3.32	0.988	2.47	0.648	4.86	0.301	3.64	0.455	2.74	0.601
	F	3.32	0.504	4.55	0.335	5.65	0.226	9.77	**0.044**	1.92	0.749
Salt addition / processed meat	**M**	3.25	0.515	4.29	0.367	1.00	0.908	3.64	0.455	9.02	0.060
	F	11.5	**0.021**	3.47	0.481	3.81	0.432	4.47	0.345	7.51	0.111
Fast and Processed Food	**M**	5.84	0.211	3.97	0.410	14.3	**0.006**	1.80	0.771	10,1	**0.038**
	F	4.34	0.361	4.98	0.289	6.01	0.198	5.14	0.272	0.84	0.932
Carbonated and Sweetened Beverages	**M**	1.35	0.852	1.22	0.873	1.42	0.839	4.15	0.385	5.36	0.252
	F	3.26	0.514	3.52	0.474	6.86	0.143	2.86	0.580	4.53	0.338
Energy Drinks	**M**	4.44	0.348	9.10	0.058	6.21	0.183	11.6	**0.020**	6.80	0.146
	F	2.56	0.633	1.80	0.771	1.95	0.744	4.09	0.392	4.57	0.334
Alcohol	**M**	4.57	0.334	0.46	0.976	1.52	0.821	2.15	0.706	4.88	0.299
	F	9.69	**0.045**	7.51	0.110	12.0	**0.016**	8.87	0.064	5.82	0.212
Smoking	**M**	1.95	0.744	3.49	0.478	9.44	0.050	6.78	0.147	4.50	0.342
	F	16.4	**0.002**	5.34	0.254	5.076	0.279	3.51	0.474	11.0	**0.025**

The consumption of fast and processed food among male students was significantly associated with type 1 diabetes **($\chi^2 = 14.3$, $p = 0.006$;** Cramer's V = 0.1537) and overweight **($\chi^2 = 10.1$, $p = 0.038$;** Cramer's V = 0.1129). The consumption of energy drinks among males showed an association with kidney disease **($\chi^2 = 11.6$, $p = 0.020$;** Cramer's V = 0.1385). Among female students, a significant association was observed between alcohol consumption and hypertension **($\chi^2 = 9.69$, $p = 0.045$;** Cramer's V = 0.1048) as well as type 1 diabetes **($\chi^2 = 12.0$, $p = 0.016$;** Cramer's V = 0.1170), while smoking was significantly associated with hypertension **($\chi^2 = 16.4$, $p = 0.002$;** Cramer's V = 0.1352) and overweight **($\chi^2 = 11.0$, $p = 0.025$;** Cramer's V = 0.1119). These findings suggest that certain lifestyle habits—such as low levels of physical activity, prolonged sitting, unhealthy dietary patterns, and the consumption of alcohol and tobacco—may be associated with an increased risk of developing cardio-renal-metabolic conditions in the student population.

Discussion

The results of the conducted study indicate multiple associations between lifestyle habits and self-reported diagnoses reflecting an increased CKM risk among the student population. Although this is a cross-sectional study that cannot establish causal relationships, the findings provide relevant insights into the potential mechanisms by which certain behaviors may influence the health status of young people.

Physical Activity, Sedentary Behavior and Health Outcomes A significant association between physical activity levels and hypertension in men suggests that physical inactivity may be one of the key risk factors for the development of high blood pressure. The physiological mechanisms supporting this include increased sympathetic nervous system tone, decreased insulin sensitivity, and impaired endothelial function, which can result in vascular dysfunction over time. In contrast, regular physical activity promotes the release of vasodilators (e.g., nitric oxide), reduces systemic inflammation, and positively affects glucose and lipid metabolism (Biernat at al., 2024, Adams et al., 2017). In women, physical inactivity was associated with elevated lipid levels and excess body weight, suggesting that the metabolic effects of inactivity may manifest earlier in female students than in their male counterparts. It is possible that hormonal status (e.g., estrogen fluctuations) modulates the body's response to a sedentary lifestyle, making women more susceptible to the negative metabolic consequences of reduced energy expenditure and increased intake of refined carbohydrates. A sedentary lifestyle was highly prevalent in both groups, and a statistically significant association was observed between sitting duration and chronic kidney disease in women (Moulin at al. 2019). Although this association requires cautious interpretation, it is possible that chronically reduced kidney perfusion due to prolonged sitting and low physical activity levels contributes to the development of functional kidney impairments. Studies suggest that sitting continuously for more than six hours a day negatively affects glomerular filtration and increases levels of inflammatory markers (Garn and Simonton, 2023).

Dietary Habits An analysis of dietary habits revealed that men more frequently consume fast food, sugary drinks, and energy drinks, which are significantly associated with higher rates of diabetes and chronic kidney disease. Energy drinks, which often contain high doses of caffeine, taurine, and sugar, can lead to blood pressure fluctuations, dehydration, and increased strain on the kidneys. Long-term consumption of such beverages is particularly risky when combined with physical inactivity and irregular sleep patterns—a common occurrence among students (Bawadi at al. 2019., Opoku-Acheampong at al. 2018). In women, the addition of extra salt to food and frequent consumption of processed foods were associated with the occurrence of hypertension (Kotopoulou at al. 2023). This association is expected, given that high sodium intake leads to fluid retention, increased blood volume, and a subsequent rise in blood pressure. Research shows that young women are often unaware of the amount of hidden sodium and salt in industrially processed foods, highlighting the need for additional education.

Alcohol and Smoking – Underestimated Risks Alcohol consumption and smoking have shown a clear association with elevated blood pressure and obesity, particularly in women (Salinas-Mandujano et al., 2023; Minzer et al., 2020). The fact that women are more sensitive to the metabolic and vascular effects of alcohol (due to a smaller distribution volume and lower levels of alcohol dehydrogenase enzymes) explains this difference. In men, these associations were not found to be statistically significant, which may be due to the higher normalization of these behaviors in the male student environment and potential underestimation of their long-term effects. Based on the obtained results, it can be concluded that both proposed hypotheses were partially confirmed. The first hypothesis (H1), which assumed that lower levels of physical activity are associated with a higher prevalence of hypertension, dyslipidemia, and overweight, was supported in part of the sample. Among male students, a significant association was observed between low physical activity and hypertension, while in female students, physical inactivity was significantly related to overweight and elevated fat levels.

The second hypothesis (H2), which posited that unhealthy dietary habits are associated with poorer self-rated health and increased CKM risk markers, was also partially confirmed. Gender differences were observed: in women, high salt intake and frequent consumption of processed food were associated with hypertension. Male students more frequently consumed fast food, sugary drinks, and energy drinks—dietary patterns that are linked in the literature to metabolic and renal risks—although these associations were not statistically significant within the male subgroup in this study.

These findings highlight the importance of gender-sensitive approaches in both research and health promotion interventions, and emphasize the need for future longitudinal studies to more precisely identify causal relationships between lifestyle behaviors and health outcomes in the student population.

Methodological Limitations of the Study It is important to highlight some methodological limitations of this study. First, data were collected through self-reporting, which increases the possibility of bias and subjective error. The cross-sectional design does not allow for a clear establishment of causal relationships. Although students from all major Croatian universities who voluntarily completed the questionnaire were included, the results cannot be generalized to the entire student population due to the convenience sample. Furthermore, a more comprehensive explanation of potential existing CKM risks in the student population would require the inclusion of additional variables related to socioeconomic status, sleep quality, and family history, which act as moderators of the relationship between behavior and health. Future research is recommended to use a longitudinal approach and include objective measures (e.g., anthropometric data, biochemical markers, blood pressure measurements) to increase the validity of the findings and facilitate a better understanding of risk dynamics during this life stage.

Practical Implications and Recommendations for Interventions The results obtained highlight the need for the implementation of comprehensive preventive programs within university environments. Such programs should include: (i) Educational workshops on healthy nutrition, the dangers of energy drinks, smoking, and alcohol consumption; (ii) Introduction of mandatory physical education and health culture courses throughout the study period; (iii) Digital tracking programs for physical activity (e.g., step-counting apps or motivational challenges); (iv) Incentives for healthy behavior – such as free or subsidized sports and recreational facilities and awarding ECTS credits for participation in public health activities; (v) Designated spaces for active teaching (e.g., classrooms with standing desks), active breaks on campuses (e.g., areas for stretching, badminton, and other recreational activities based on faculty resources and existing infrastructure).
Furthermore, it is recommended to systematically monitor the health status of students through regular preventive screenings, enabling early detection of risks and timely interventions.

Conclusion

This study highlights the significant presence of unhealthy lifestyle habits among students, including low levels of physical activity, prolonged sitting, poor dietary habits, and the consumption of alcohol and tobacco. Clear gender differences were observed in certain behavioral patterns, which should be taken into account when designing preventive programs. Gender differences in health outcomes likely reflect both biological factors (such as hormonal profiles and body fat distribution) and lifestyle differences, underscoring the need for gender-specific preventive strategies in promoting student health. The results emphasize the importance of early education and the promotion of healthy lifestyle habits within the student population to reduce the risk of developing cardio-renal-metabolic diseases later in life. Furthermore, it is necessary to develop concrete policies and systematic interventions aimed at encouraging physical activity, healthier diets, and the reduction of risky behaviors among students. Collaboration with various professionals—such as nutritionists, physiotherapists, and psychologists—is recommended to ensure a holistic approach to student health. Future research should focus on the longitudinal monitoring of these habits and the examination of their impact on objective indicators of cardio-renal-metabolic risk.

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Empirical Evaluation of the Validity of Near-Death Experience Claims and Assessment of Neuropsychiatric and Motor Deficits Following Cardiac Surgery in Patients Undergoing Planned Circulatory Arrest – Study Design

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This article was submitted to RAD CASA - Medical Sciences as the original article

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 20 May 2025
Accepted: 29 May 2025
Published: 25 June 2025

Citation:
Filipan A, Pavlović P, Hostić V, Maričić M, Silić A, Josipović J, Darko Gabrić I. Empirical evaluation of the validity of near-death experience claims and assessment of neuropsychiatric and motor deficits following cardiac surgery in patients undergoing planned circulatory arrest – study design

567=70-71 (2025): 44-50
DOI: 10.21857/yrvgqt3w79

Abstract

Introduction: Near-death experiences (NDEs) have been widely reported across cultures, yet their underlying causes remain debated. This study design focuses on prevalence, phenomenology and psychological impact of NDEs in patients undergoing planned circulatory arrest during cardiac surgery. The study also assesses the neuropsychiatric and cognitive consequences of such procedures using a mixed-methods approach.
Materials and Methods: The study uses a convergent parallel mixed-methods design, combining both qualitative and quantitative data collection. Participants will undergo neuropsychiatric and motor tests (MOCA, TNT-A and B) pre- and post-surgery to assess cognitive and motor deficits. In addition, psychometric instruments, including the Greyson NDE Scale, Mysticism Scale, and Purpose in Life Test, will be administered to evaluate the emotional and psychological aspects of experiences. Patients who report NDEs will participate in semi-structured interviews, while medical staff will be surveyed for objective data regarding the surgery. The VR simulation of the NDE experience will be offered to patients who consent, allowing further exploration of cases of out-of-body experiences.
Results: The study design aims to determine the prevalence of NDEs following planned circulatory arrest, the correlation between the duration of circulatory arrest and memory freshness, and the psychological impact of these experiences. It is hypothesized that at least 10% of patients will report NDEs, and some will report out-of-body experiences (OBEs). Additionally, it is expected that patients who score higher on the Greyson NDE Scale will show greater scores on mysticism and purpose in life tests. Data will be analysed using correlation tests, t-tests, and thematic analysis of interview responses.
Conclusions: This study aims to provide new insights into the prevalence and psychological effects of NDEs in cardiac surgery patients, with potential implications for understanding consciousness and the experience of death. By using a combination of standardized psychometric tools and qualitative interviews, this research will contribute to the scientific understanding of NDEs while ensuring participant safety and data integrity.

Keywords: near-death experience, neuropsychiatric deficit, motor deficit, cardiac surgery, planned circulatory arrest

Sažetak

Empirijska evaluacija valjanosti tvrdnji o iskustvima bliske smrti iprocjena neuropsihijatrijskih i motoričkih deficita nakon operacije srca kod pacijenata koji se podvrgavaju planiranom cirkulatornom nastoju – dizajn studije

Uvod: Iskustva bliske smrti (NDE) su često spominjana u različitim kulturama, no njihovi temeljni uzroci i dalje su predmet rasprave. Ovaj studijski dizajn usmjeren je na prevalenciju, fenomenologiju i psihološki utjecaj NDE kod pacijenata koji su podvrgnuti planiranom zastoju cirkulacije tijekom operacije srca. Studija također procjenjuje neuropsihijatrijske i kognitivne posljedice takvih postupaka korištenjem pristupa mješovitih metoda.

Materijali i metode: Studija koristi konvergentni paralelni dizajn mješovitih metoda, kombinirajući kvalitativno i kvantitativno prikupljanje podataka. Sudionici će prije i poslije operacije proći neuropsihijatrijske i motoričke testove (MOCA, TNT-A i B) kako bi se procijenili kognitivni i motorički deficiti. Osim toga, bit će primijenjeni psihometrijski instrumenti, uključujući Greysonovu NDE skalu, Skalu misticizma i Test svrhe života, kako bi se procijenili emocionalni i psihološki aspekti iskustava. Pacijenti koji prijave NDE sudjelovat će u polustrukturiranim intervjuima, dok će medicinsko osoblje biti anketirano radi dobivanja objektivnih podataka o operaciji. VR simulacija NDE iskustva bit će ponuđena pacijentima koji pristanu, što će omogućiti daljnje istraživanje slučajeva izvantjelesnih iskustava.

Rezultati: Cilj studije je utvrditi prevalenciju bliskosmrtnih iskustava (NDE) nakon planiranog zastoja krvotoka, korelaciju između trajanja zastoja krvotoka i svježine pamćenja te psihološki utjecaj tih iskustava. Pretpostavlja se da će najmanje 10% pacijenata prijaviti NDE, a neki će prijaviti izvantjelesna iskustva (OBE). Osim toga, očekuje se da će pacijenti koji postižu više rezultate na Greysonovoj NDE ljestvici pokazati veće rezultate na testovima misticizma i svrhe u životu. Podaci će se analizirati pomoću testova korelacije, t-testova i tematske analize odgovora u intervjuima.

Zaključci: Cilj ove studije je pružiti nove uvide u prevalenciju i psihološke učinke NDE kod pacijenata na operaciji srca, s potencijalnim implikacijama za razumijevanje svijesti i iskustva smrti. Korištenjem kombinacije standardiziranih psihometrijskih alata i kvalitativnih intervjua, ovo istraživanje će doprinijeti znanstvenom razumijevanju NDE-a, a istovremeno će osigurati sigurnost sudionika i integritet podataka.

Ključne riječi: iskustvo bliske smrti, neuropsihijatrijski deficit, motorički deficit, operacija srca, planirani cirkulatorni zastoj

1. Introduction

1.1. Statement of compliance

This clinical trial will be conducted in full compliance with the protocol, the International Council for Harmonisation Good Clinical Practice (ICH GCP) guidelines, and all applicable regulatory requirements of the Republic of Croatia and the European Union, including Regulation (EU) No. 536/2014 on clinical trials and the Croatian Medicines Act, as well as the General Data Protection Regulation (GDPR) (1, 2, 3, 4).

All participating institutions are required to obtain approval from a competent Ethics Committee (EC) prior to initiating the trial, including approval of the protocol, informed consent form(s), and any recruitment materials. Any amendments to the protocol or consent documents must also be submitted for EC approval before implementation.

The study design is based on fundamentals of Parnia's 2014 clinical study conducted in the United Kingdom, known for its strict ethical and regulatory standards (5). The design has been carefully adapted to ensure compliance with EU and Croatian regulations while maintaining a high standard of participant safety, rights, and data protection.

The principal investigator confirms that no deviations from or changes to the protocol will be implemented without prior agreement from the relevant regulatory authorities and documented approval from the Ethics Committee, except when necessary to eliminate immediate hazards to trial participants. All study personnel involved in the conduct, oversight, or management of the trial will have completed training in Human Subjects Protection and ICH GCP guidelines (1, 6).

The protocol, informed consent form(s), recruitment materials, and all other participant-facing documents will be submitted to the Ethics Committee for review and approval. Approval of both the protocol and consent form(s) must be obtained prior to enrolling any participant. Any amendments to the protocol or consent materials will require prior Ethics Committee approval. A determination will be made as to whether re-consent is necessary for participants who were previously enrolled under an earlier version of the consent form.

1.2. Protocol summary

This study explores the empirical basis of near-death experiences (NDEs) using a convergent parallel mixed-methods design, combining qualitative and quantitative approaches (7). The study aims to collect and analyse data from adult individuals (18+) in Croatia who self-identify as having experienced an NDE after cardiac surgery with a planned circulatory arrest (8, 9). Participants will first undergo the MoCA test and the Trail Making Test Parts A and B (TMT-A and B) before and after surgery, following informed consent, to assess potential neuropsychiatric and cognitive deficits (18, 19). In addition to post-operative assessments, participants will complete in-person questionnaires with the research team. Those who report a near-death experience (NDE) will subsequently be invited to participate in semi-structured interviews. The quantitative component involves validated psychometric instruments (e.g., Greyson NDE Scale, Mysticism Scale, Purpose in Life Test) (10, 11, 14). Qualitative data will be analysed using thematic analysis (12). Recruitment will be conducted through informing target group patients before cardiac procedure. The study includes informed consent, baseline data collection and individual interviews scheduled at participants' convenience. Follow-up session including virtual reality depiction of NDE will be available only for interested patients upon providing informed consent. That will be done to offer validation and show individuality of their experiences, to support long-term transformational effects (13, 20).

1.3. Introduction

Near-death experiences (NDEs) have been reported across cultures and clinical contexts, yet their origins—whether neurobiological, psychological, or transcendent—remain contested (5). This study addresses gaps in Croatian research on NDEs by empirically analysing their occurrence, phenomenology and psychosocial impact. The rationale is grounded in the need for scientifically rigorous, culturally contextualized data on NDEs to inform interdisciplinary understanding. The study is adapted from a 2014 UK model known for ethical and methodological standards (5). It builds on prior exploratory and phenomenological research while incorporating standardized instruments for psychological profiling (10, 11). Literature suggests links between NDEs and lasting changes in personality, spirituality, and well-being, but Croatian empirical data are lacking (13). This research aims to provide insight into such transformations, enabling a better understanding of NDEs from both scientific and existential perspectives (15).

1.4. Risk/benefit assessment

Risks are minimal and primarily psychological, stemming from potential emotional discomfort during interviews as participants recall potentially traumatic or deeply personal experiences (13). These will be mitigated through interviewer training, the right to withdraw, and resource referrals. There are no physical risks. Benefits include contributing to underexplored scientific knowledge and potentially therapeutic effects of meaning-making through narration. Societal benefit lies in advancing interdisciplinary understanding of consciousness, process of dying and human transformation.

1.5. Objectives and endpoints

Objectives of this study are following:

a) Determine the prevalence of NDEs in patients after planned circulatory arrest using the Greyson Scale.
b) Analyse patients' experiences, including observations of the surroundings, present individuals, and events during the cardiothoracic surgery (out-of-body experience (OBE), transformation experience).
c) Correlate the time spent in planned circulatory arrest and the time of the survey with the freshness of memories to eliminate bias.
d) Quantify the emotional and psychological aspects of experiences using Likert scales.
e) Identify neuropsychiatric and cognitive consequences in patients using the MOCA test and TNT-A and B tests before and after surgery.
f) Compare patients' subjective experiences with objective statements from medical staff and the environment.

2. Materials and Methods

2.1. Study design and rationale

This study proposes a prospective, multicenter, mixed-methods investigation of near-death experiences in adult survivors of planned cardiac arrest. The study uses a convergent parallel mixed-methods design (7). This allows simultaneous collection of qualitative (interview) and quantitative (survey) data for integrated interpretation. The design is non-interventional and exploratory. It is not blinded nor randomized, as the subject matter (personal experiences of NDEs) does not permit such designs. The qualitative arm includes semi-structured interviews (~20–40 minutes) with participants, analysed via thematic analysis (12). The quantitative arm includes standardized psychometric tools. These include the Greyson NDE Scale, Mysticism Scale, Purpose in Life Test. Data will be triangulated to identify overlapping constructs and validate themes. The rationale lies in capturing the depth of individual NDEs while situating them within psychological and physiological constructs. After pre-surgery neuropsychiatric and motor tests and post-surgery assessments, NDE information will be collected only from the first interview, minimizing re-exposure to reduce discomfort and memory distortion (15). The study will be conducted in person. Study design scheme is presented in Figure 1.

2.2. Study population

Inclusion criteria: adults (18+), fluent in Croatian, who report having experienced an NDE (per test answers) and can provide informed consent.

Figure 1. Timetable of gathering research data

Exclusion criteria: individuals with active psychosis or severe cognitive impairment that prevents informed participation, decline of participation, unconscious for longer than one month period and death.

Participants will be recruited through informing pre-procedure. Lifestyle factors in this prospective study are limited, as participants are included shortly after their involvement in the research and provide real-time data, within a window that minimizes susceptibility to suggestibility or memory distortion. Participants are expected to remain hospitalized for a brief period, ranging from one to four weeks. Screen failures may occur if participants do not meet inclusion criteria after initial contact or if their narratives fall outside the NDE construct (5). Emphasis will be placed on voluntary participation and narrative authenticity.

2.3. Study intervention

There is no clinical intervention. The intervention is the act of participating in the study via:

- completing pre- and post-operative tests to assess neurocognitive function: MOCA Test, TNT-A and B;
- completing surveys consisting of validated scales: Greyson NDE Scale, Mysticism Scale, Purpose in Life Test;
- participating in a recorded semi-structured interview and
- optionally providing consent to virtually recreate audio-visual component OBE during NDE.

Participants can choose to engage in neither, some or all components. Interviews will be conducted online/in person and audio-recorded with consent. There is no medication or physical procedure involved. Adherence is ensured via digital reminders and participant choice of timing. Participants may withdraw at any time without consequences. Discontinuation involves deletion of unprocessed data upon request. For patients whose experiences align with NDEs (especially with OBE elements), medical staff will be surveyed for information about the surgery, patient behaviour and comparisons with reported experiences. Physiological parameters such as ECG, acid-base balance, arterial oxygen levels, and drugs administered during planned circulatory arrest will also be considered. The structured interview will focus on NDEs and OBEs, including descriptions of audiovisual perceptions. In the operating room, predefined visual and auditory stimuli will be placed in areas visible only from the ceiling, similar to Parnia's 2014 study (5). These are expected to be reported by patients. Test probes will also be placed in locations visible from the patient's position to distinguish observations from lucid states before and after surgery.

3. Results

3.1. Assessments and safety

Safety risks are limited to potential emotional discomfort. Interviewers will be trained in trauma-informed interviewing (16). Participants will be advised of their rights and provided with mental health resources (17). All adverse events (e.g., emotional distress) will be noted and, if severe, referred to appropriate care. Data will be pseudonymized to ensure confidentiality. Interview and survey responses will be stored securely and monitored for data completeness. Unanticipated problems will be addressed case-by-case, in consultation with the Ethics Committee if required.

3.2. Statistical plan

3.2.1.Hypotheses

- Patients undergoing cardiothoracic surgery will exhibit neuropsychiatric and motor deficits post-surgery compared to their pre-operative condition.
- More than 10% of patients who undergo cardiothoracic surgery will report near-death experiences (NDEs).
- More than 2% of patients will report out-of-body experiences (OBEs) related to the period of clinical death or reduced consciousness.
- Participants with higher Greyson Scale scores will show greater scores on mysticism and purpose in life measures.

3.2.2. Study power analysis

To ensure high statistical reliability, the study will be powered at 90% with a significance level of $\alpha = 0.01$. Based on this, a minimum of approximately 238 patients is required to estimate a 10% prevalence with high confidence, and over 1,100 patients are needed to detect medium-sized group differences with sufficient power, assuming that 10% of the total sample will report NDEs. To reach these numbers, the study will be conducted across multiple hospitals, enabling broader recruitment and inclusion of a diverse patient population. The qualitative component will include in-depth, semi-structured interviews with approximately 6 to 15 patients who report NDEs, which is considered sufficient to achieve thematic saturation and provide a nuanced understanding of their subjective experiences. This complementary design will allow for both statistically grounded findings and rich, experiential insights into consciousness following planned cardiac arrest.

3.2.3.Sample size calculation

- Qualitative: ~238-1100 participants until thematic saturation.
- Quantitative: ~6-15 participants, enabling correlational analyses with adequate power.

3.2.4.Populations for analysis

All adult participants who undergo the procedure of heart operation with planned circulatory arrest will be included in quantitative analysis. For qualitative analysis, interview transcripts will be analysed for thematic saturation.

3.2.5.Statistical Methods

Correlations (Pearson/Spearman), t-tests, ANOVA and multiple regression may be used to test hypotheses. NVivo or similar

software will be used for qualitative coding. Integration will occur via triangulation matrix to link quantitative profiles with qualitative themes.

4. Discussion

4.1. Why planned circulatory arrest

The planned circulatory arrest closely resembles the resuscitation process and is ideal for studying NDEs because it occurs unexpectedly and without external influences such as medications. In contrast, the planned arrest does involve the administration of various drugs, and therefore, we can expect the data to differ somewhat from those obtained in cases of spontaneous cardiac arrest. It is expected that with a sufficient number of study participants, more than 10% of them will report having had a NDE. However, their experiences may qualitatively differ from those in a classical setting (since drugs are often associated with negative NDE experiences), or participants may not remember them implicitly due to the influence of medications. This is the focus of the study.

4.2. Regulatory, ethical, and study oversight considerations

Informed consent will be obtained before participation. Ethical approval will be sought from a competent EC prior to study start. Participants will be informed of their rights, including the right to withdraw and data protection per GDPR. All data will be anonymized or pseudonymized. The principal investigator will oversee compliance, and all study staff will be trained in ethics and data protection. There is no external monitoring, but internal oversight will ensure adherence to protocol and ethical conduct. Any protocol amendments will be submitted for EC approval prior to implementation.

4.3. Quality assurance and data management

Data will be stored on secure platforms. Interview data will be recorded, transcribed, and coded in anonymized form. Surveys will be stored in encrypted format. Only authorized personnel will access data. Protocol deviations (e.g., incomplete surveys, withdrawn consent) will be documented. Regular checks for data integrity and quality will be conducted by the PI.

4.4. Publication and other policies

Findings will be submitted to peer-reviewed journals and shared at academic conferences. Anonymized datasets may be shared upon request for secondary analysis, with EC approval. Any conflicts of interest will be disclosed. Authorship will follow ICMJE criteria.

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The Meaning of Life and the Process of Self-Actualization

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OPEN ACCESS

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This article was submitted to RAD CASA - Medical Sciences as the review article

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 23 April 2025
Accepted: 29 May 2025
Published: 25 June 2025

Citation:
Salopek Z, Demarin V. The Meaning of Life and the Process of Self-Actualization

567=70-71 (2025): 52-59
DOI: 10.21857/yvjrdcdvzy

Abstract

This paper explains and analyzes the relationship between the meaning and purpose of life and emotional maturation as a path toward self-actualization. In addition, it presents a new model of self-actualization, a new model of maturity levels, connecting them through the lens of emotional maturation - consciousness. Central to this process is the role of the ego, viewed as emotional inexperience that creates a false identity to compensate for feelings of inferiority created by this inexperience. Emotional maturity arises from confronting ego-driven fears and challenges, which foster learning and the development of self-actualization skills, including selflessness, perseverance, acceptance, and forgiveness. Furthermore, the paper critiques existing maturity models and proposes a new map of emotional maturity levels. This progression highlights that true maturity involves silencing the ego thru emotional knowledge and embracing inner balance.

Keywords: Meaning and purpose of life. Self-actualization. Ego. Emotional maturation.

Sažetak:

Smisao života i proces samoostvarenja

Ovaj rad objašnjava i analizira odnos između smisla i svrhe života i emocionalnog sazrijevanja kao puta prema samoostvarenju. Osim toga, predstavlja novi model samoostvarenja, novi model razina zrelosti, povezujući ih kroz prizmu emocionalnog sazrijevanja - svijesti. Središnja uloga ovog procesa je uloga ega, promatranog kao emocionalno neiskustvo koje stvara lažni identitet kako bi kompenziralo osjećaje inferiornosti stvorene tim neiskustvom. Emocionalna zrelost proizlazi iz suočavanja sa strahovima i izazovima vođenim egom, što potiče učenje i razvoj vještina samoostvarenja, uključujući nesebičnost, ustrajnost, prihvaćanje i oprost.

Nadalje, rad kritizira postojeće modele zrelosti i predlaže novu kartu razina emocionalne zrelosti. Ovaj napredak naglašava da istinska zrelost uključuje utišavanje ega kroz emocionalno znanje i prihvaćanje unutarnje ravnoteže.

Ključne riječi: Smisao i svrha života. Samoostvarenje. Ego. Emocionalno sazrijevanje.

Introduction

The meaning of life is one of humanity's most essential and complex questions. Nevertheless, there is an Absolute Truth answer to that question, as to any other, if we have enough experience to understand it. It is this topic that the present paper explores, the Absolute Truth for all the questions we will be addressing here.

It is important to highlight that Absolute Truth is defined as a statement that is true at any time and in any place, always true regardless of the circumstances. It is a fact that cannot be altered. In essence, the *truth* itself is absolute; otherwise, it is not the *truth*, it is perpective. Therefore, in the remainder of this paper, we will refer to it simply as *truth*.

At one time, a crucial need arose in medicine to model and uncover the *truth* about human anatomy, leading us to dissect bodies in order to understand how they function, what causes various processes, and which effects follow. Even before medicine, we began modeling the *truth* of our sky and stars. Today, humanity has reached the point where it needs to model the *truth* of our inner and outer worlds. It is this model that the present paper outlines, covering all the details of the self-actualization process and providing a map of the human inner world.

For a long time, it was mistakenly believed that the Earth was the center of the universe. The disproving of this misconception used to lead to strong resistance and violence until the society realized that the *truth* was actually different, confronted this misconception, and changed it in scholarly texts. Today, there is a misconception that our external world is of central importance and that our need to prove ourselves and play a role in it is the center of everything. At least, our surroundings and our ego try to convince us of this. Challenging this misconception also generates a lot of resistance. This resistance will persist until, as a society, we recognize another *truth*—that our inner world is the center where we are important and where our maturation and self-actualization take precedence. It is also the *truth* that life, the external world, our social relationships, work, or family serve merely as a stage, a training ground for accumulating experiences and exercising our maturity.

We are all aware that the human desire to prove the existence of *truth* of us humans lies beyond the reach of our limited speculative minds. Indeed, the *truth* about ourselves and our lives cannot be proven; we will need to agree on this as a species. If it could be proven, it would simply be too easy. The first person to prove it would set a path that others would merely follow. In this way, a consensus is required—a shared experience and mutual understanding that will lead to a common knowledge and a path for others.

The meaning of life

The need to mystify the meaning and purpose of life stems from pure ambiguity, and the *truth* about what the meaning of life is is so simple that it can be summed up in one word: experiences.

The meaning of life for us humans is to gather experiences and mature through the process of self-actualization. As such, it is collective in nature and predetermined for us humans, who in this world of duality have, on one hand, an irresistible need—almost a task—to explore and develop, to create and connect. On the other hand, there is a fear of inexperience and the unknown, of change or pain. By fulfilling these needs, overcoming fears, and exploring the unknown, we collect new experiences (Frankl, 1946; Rogers, 1969). To explain the meaning of life and self-actualization in detail, we must clearly distinguish what types of experiences exist, and what experience truly is.

In line with our external and internal world, our experiences can be categorized as external and internal. All our needs provide the impetus that drives us to seek experiences in the external world, while our conscious and unconscious fears that manifest as *ego challenges*, give us the opportunity to overcome them and thereby gain internal experiences or self-actualization skills such as responsibility, acceptance, forgiveness, and others.

We gain experience when something becomes truly unimportant to us. Traveling a lot, going out, or constantly proving ourselves and seeking attention does not mean we have experience. With these activities we explore and study that experience, and we truly comprehend that experience when the traveling, nightlife, or proving ourselves and seeking someone's attention become simply unimportant. It is exactly through the gathering of these experiences that we mature and self-actualize. We have experienced all of this and can do it, but we do not want to; we do not see the value in it; it becomes unimportant (Salopek, 2023).

The importance and significance of experience are entirely the same at the social level as they are for the individual due to our interconnectedness. This is best illustrated by the fact that it took humanity approximately a million years to understand the experience of ordinary fire, which then enabled further development of our specie. For a million years, we as a society struggled to comprehend fire, and the moment we grasped that experience, it became again - unimportant. No one talks about fire anymore; it is irrelevant and unnecessary, even amusing to marvel at it (Colman, 2003). However, it was a significant step in the development of us humans as a species, because a species self-actualizes in the same steps as an individual, thereby proving our interconnectedness.

Self-actualization

Self-actualization was first mentioned in 1943 in a paper on the hierarchy of human needs by American psychologist Abraham Maslow. However, it only referenced a few internal needs, leading to a misinterpretation of self-actualization as merely one of the needs, rather than a process. We achieve self-actualization by accumulating experiences, and these experiences are fulfilled needs, which fundamentally makes them the same topic. However, self-actualization in the context of meaning and purpose in life is much more complex. A new model of the self-actualization process through the fulfillment of needs and the gathering of experiences can be found in Figure 1 (Salopek, 2023).

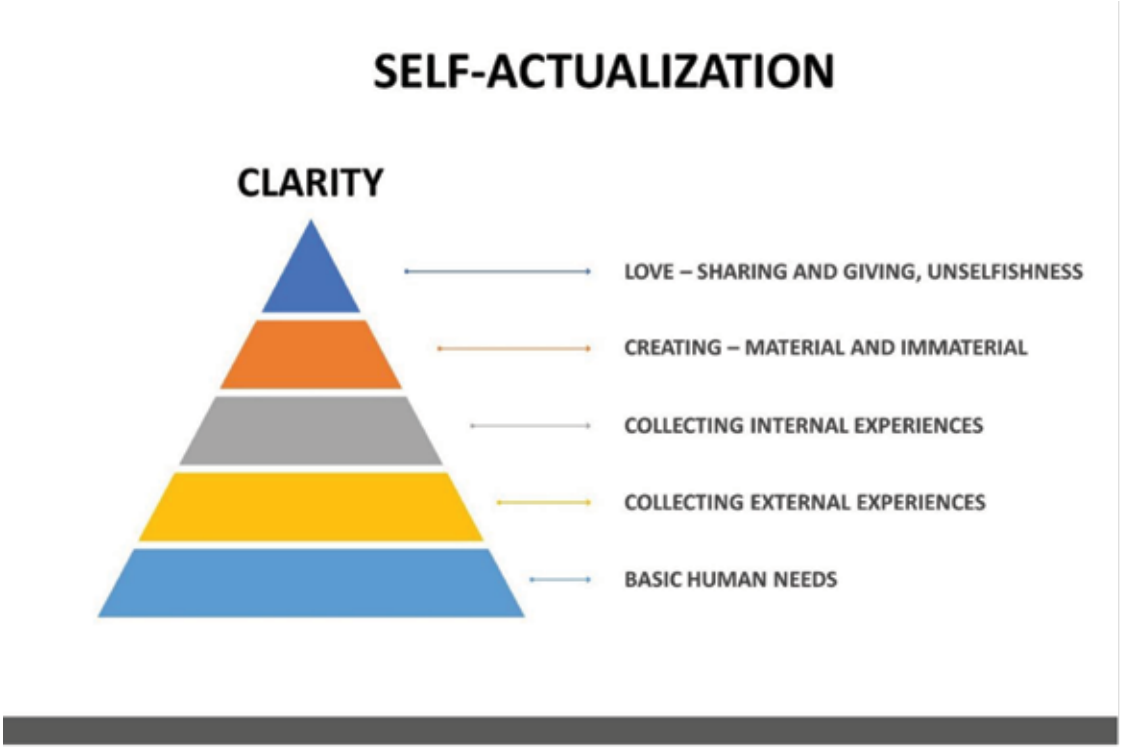

Figure 1. Pyramid of Self-Actualization

The base of the self-actualization pyramid is indeed defined by fundamental human needs, such as physical and psychological safety, which are prerequisites for existence and maturity. If basic needs for food and water are not met, or if one is physically or psychologically threatened, all attention and energy will instinctively be directed toward survival, rather than the gathering of experiences, introspection, creation, and personal growth (Maslow, 1982). This level teaches us the skill of survival.

Once these needs are satisfied, individuals move to the next level of the pyramid, seeking experiences from the external world based on what they observe around them. There is a strong desire for experiences that align with their maturity and previously gathered experiences, focusing instinctively on new experiences that they lack in the external world, such as playing, riding a bike or driving a car, first kisses, belonging, relationships, material possessions, nightlife, knowledge, first jobs, parenting, or traveling. Human needs or impulses exist to drive us toward the gathering of the experiences we lack. Moreover, unwanted events and traumas, such as a broken arm or divorce, are also experiences. With the passage of time, these events lose their negative emotional connotation and remain as mere experiences, since even children cannot learn to walk without falling. However, up to this point, animals can do the same since they also experience the basic needs such as birth, survival, work, finding their place in the social hierarchy, reproduction, and death. What they cannot do, however, is explore their inner world, which is the next step in the process of self-actualization (Salopek, 2023).

In the middle of the pyramid, the individual focuses on inner experiences, consciously or unconsciously turning toward the fulfilling of their internal needs and overcoming of fears. Our internal needs for respect, attention, validation, trust, and similar sentiments stem from immaturity and a lack of experience. As one matures, these needs diminish in significance because the experiences gained help alleviate the fears that drive them. Fears are the root of our immaturities and weaknesses, providing challenges that allow us to acquire new skills and experiences while exploring our inner world (Segrin, 2007).

For instance, if a person has never possessed many material items, that lack of experience may lead to a strong desire for them. From this perspective, the individual might believe that owning material things affirms their worth and importance. As they accumulate more possessions, they gain more experience. However, true understanding comes when they realize that these items are ultimately unimportant. Only then can they say they have learned the skill of letting go and detachment. If a person still feels a need for these items or concepts, they have not yet grasped that experience and cannot progress along the path of self-actualization (Kardas et al., 2019).

Further on, due to the trauma and negative experiences we have endured, we often develop resentment towards the person or event that caused that trauma. This trauma and the accompanying resentment serve as experiences that aim to teach us a necessary skill in our maturation process: forgiveness. All our fears and the consequent character weaknesses caused by mere inexperience exist to challenge us and teach us the skills of self-actualization.

After accumulating enough external and internal experiences, a person transitions from being a survivor to a creator—turning their focus to the act of creation itself. On the second-to-last level of the pyramid, we find creation, both material and immaterial. The specific object of creation is not important, be it art, business, a book, a family, a film, an application, or a house. What matters is our dedication to the act of creation itself. By creating, we present something external to ourselves and divert attention from how we feel in the process. Paradoxically, this focus on creation allows us to delve deeper into ourselves, gathering experiences, maturing, and growing until we realize the value of giving—love (Salopek, 2023).

At the top of the pyramid of self-actualization lies love, which is portrayed as an individual streght and skill developed through experience and maturity, rather than an inherent right obtained at birth. Love is characterized by unconditional giving and sharing of experiences acquired on the journey to self-actualization, both with loved ones and strangers. Unlike the meaning of life, which is collective in its nature, the purpose of life is individual and pertains to the experiences one must gather at any given time. The highest purpose in life is to share these experiences gained through maturity, akin to how a mother selflessly shares her experiences with her child. Interestingly, the need to share and give is also egoistic if it is conditional; true love cannot have conditions. If one still feels a need for something, it indicates a lack of mastery over the skill of love. Thus, to truly love, we can do whatever we want, but we must not be in need of it, since the need itself will prevent us from mastering the skill of love. When we need nothing and learn to love, we automatically enter the present moment (De Karoli, 2014).

At the end, love will lead us to clarity. This is the reward for a fulfilled meaning of life, rather collected experiences. Clarity is also both internal and external. In a moment of complete internal clarity, nothing from the external world will be able to affect us or our inner world anymore, as we have fulfilled all needs, overcome all irrational fears, and taken control over ourselves (Kardas et al. 2019).

External clarity is the realization that life is not a chaotic and random sequence of events but that there are specific laws, such as duality or cause and effect, that govern this school for gathering experiences we call life. These laws help us overcome our fears by providing life challenges, and it is up to our experience and maturity whether we interpret them as problems or assistance (Salopek, 2023).

The key to self-actualization and the meaning of life lies in experiential, emotional understanding of what is important and what is not. From our own inexperience fears are created. If we, for example, dont have experience of beeing alone, fear of loneliness will arise. From all inexperience and fears we develop a deep sense of unimportance – feeling of inferiority, leading to a need to feel important. Consequently, we unconsciously create an identity that provides us that importance. Based on the dual "*fight or flight*" program (Cannon, 1932), we either flee from this need to be important by pleasing others, becoming passive, or even resorting to self-destructive behavior, or we constantly seek validation, impose ourselves, or even act aggressively to affirm our own importance and identity. This deep sense of unimportance drives people to addictions as substitutes for love – strength and skill. All human-created addictions essentially serve to numb this deep feeling of insignificance or provide a false sense of strength and importance (Kozarić-Kovačić et al., 2024).

To achieve a balance between these two extremes through experience, we must first experience both "extremes" in this world of duality before realizing completeness and self-actualization. The most inexperienced individuals initially flee from everything because they unconsciously feel very insignificant and are actually running away from being important, both to themselves and to others. When emotional suffering compels us to explore our own importance, we begin to prove ourselves, seek attention, and collect experiences to demonstrate our value to ourselves and others. That is why emotional suffering exists, because without it who would even begin to explore their inner word? Who would deal with their fears, passiveness, or selfishness if there was no emotional pain to nudge us?

As we accumulate more experiences, we start to realize the insignificance of those same experiences. Our diplomas and positions, proving ourselves and seeking attention, mingling and excitement, travel and money become unimportant to us. Ultimately, we realize that we become insignificant to ourselves. In this realization of our own insignificance, we begin to discover our own infinity and strength, self-confidence and clarity, joy and love, the power of simplicity, and finally, our importance once again. This time, however, that feeling of importance arises not from weakness and validation, but from complete acceptance, strength, and clarity.

Thus, our entire lives and all experiences serve to help us understand that everything is unimportant, including ourselves, only to realize that we are so important that life is made just for us so we may collect experiences. It may sound paradoxical, but the understanding of this law lies in the insight itself, which follows the same pattern throughout life. Given that paradoxicality always involves returning to the starting point based on experience, I have termed it the *paradox of the circle*.

The Paradox of the Circle

The paradox of the circle is best explained through a story. I will use the metaphor of a person fishing in a small town who has never left it. This person fishes out of inexperience and fear of everything that lies in the unknown. One day, feeling bored with safety, he decides to explore the unknown and confront that fear. He sets out into the world, discovers the lights of the big city, and turns to the gathering of external experiences amidst that abundance. Amazed by the glamour, this person thinks he will never return to the small town because there is nothing left for him there. Once he experiences that abundance and realizes its insignificant nature, he matures and turns to his own inner world, questioning the importance of the external world. Upon gaining clarity about the unnecessary glamour, and a waste of time spent studying that experience, he turns to creation, but for the benefit of all, including himself. After feeling immense satisfaction in his creation, he wants to share it with others. Recognizing the importance of sharing, he also grasps simplicity and, driven by this new motivation, returns to his small town to fish and share his experiences of the big city and his realizations. Now this person is doing exactly the same thing, fishing, as he used to when he started his journey of self-discovery, but now he is doing it guided by a completely different motivation—insight and clarity, rather than fear and inexperience (Norrish et al. 2011).

By the same logic and *paradox of the circle*, a child experiences various experiences while growing up, seeking their own identity but quickly changing identities by altering their interests. These changes occur rapidly because there are no limitations in the environment, which easily accepts changes in children's interests and identities. As we grow older, it becomes increasingly difficult to change, and we establish an identity that suits us more or less. The less mature and inexperienced we are, the greater our need for identity, making it harder to discard it and seek our path in the unknown.

The path to realization lies in discarding our own identity and returning to the starting point, where we, as children, curiously and easily changed identities because we effortlessly accepted our own insignificance. Thus, throughout life, we must gather experiences, building our own identity and a sense of self-imposed importance, just to gain the strength, courage, and maturity to discard all identities. In that own insignificance, we discover who we truly are. We must become no one to be able to be everyone, thereby uncovering our true selves. (Norrish et al., 2011).

It is our motivation that will reveal to us which point of this circle we are at—the reason «why?» we do something. Overall maturity and self-actualization are defined only by our motivation. For everything we do, we will uncover our motivation by asking ourselves a simple question «why?». The answer to the question «why?» will define our motivation and, consequently, our maturity.

Therefore, *it is not important what we do, but only why we do it.*

Do we do—or not do—something out of fear and inexperience, or out of clarity and strength? The answer to this question will define our maturity and, consequently, our entire life. (Colman, 2003).

Due to our interconnectedness as individuals, we, as a society, mature and self-actualize along the same path as individuals. Throughout history, we had to go through a long and difficult first step of self-actualization, which is physical and psychological safety. It took thousands of years for us as humans, and society as a whole, to develop sufficiently so that this is no longer a topic. Naturally, we speak at the level of all humanity, not locally, where there are still many parts of society that have not even surpassed the first step. After that step, again in this world of duality, the Western part of humanity has gone to one extreme, focusing on experiences in the material world and proving themselves within it, consequently bewildered by those who seek answers in their inner world (Colman, 2003). In doing so, they unconsciously neglect the study of self-actualization skills such as silence, forgiveness, acceptance, letting go, or selflessness. In the same imbalance, the Eastern part of humanity is oriented toward the other extreme, exploring their inner world and how this life functions. In the same ignorance, they are astonished by those who seek answers in the material world, thereby unconsciously neglecting the skills such as new experiences, determination, taking responsibility, persistence, or discipline.

In this process, both worlds create and develop unaware of the fact that for the final step—the most difficult and mature, the realization of love—it is necessary to incorporate and recognize both poles before we become whole, and understand personal insignificance and the notion that no one is more important than the other, and thus that everyone is important. By understanding the skill of love, we achieve emotional maturity as individuals and as a society. (Salopek, 2023).

Emotional maturity

First of all, it is important to note in terms of terminology that emotional maturity and maturation are called personal growth and development by some authors, while some call them spirituality and the search for the true self, whereas some call these concepts awareness or personal transformation. The *truth* is that these are all synonyms, and we will use the term maturity because it is the most complete, taking into account the meaning of life, the process of self-realization and the map of the inner world. Awareness is also a plausible term because it is a complete synonym, much more popular, and therefore acceptable.

All experiences lead us to emotional maturity, i.e. self-actualization. The real core and point of emotional maturity is the inner balance achieved by facing one's own needs and fears that manifest through ego challenges and consequently learning the skills of self- actualization.

Ego is actually our overall emotional ignorance – inexperience. This creates our deep feeling of inferiority – unimportance, that we try to compensate by creting identity that will give us importance – false identity (Winnicott, 1971). The more experienced and mature we are, the less things and ideas are important to us, and with that we are actually breaking down our identity so that

through experience we return to insignificance, but this time out of strength, not fear.

However, if there were no ego, there would be no fears and challenges to overcome, and thus there would be no learning and maturing (May, 1980; Horney, 1950). Our ego and its challenges are not put there just to cause us suffering, they are given to us so that we can gain experiences and learn the skills of self-realization - fulfilled needs and skills of selflessness, letting go, silence, perseverance, understanding, growth, letting go, determination, accepting reality and yourself, and forgiveness.

Our ability to change and learn new emotional skills by confronting our fears caused by inexperience is actually our emotional intelligence. It is made out of our consciousness, conscience and courage. By the same logic, it wants to teach us the skills of responsibility, proper motivation and discipline (Salovey, Mayer, & Brackett, 2007; Goleman, 1995; Bar-On, 2005). Therefore, our emotional intelligence is actually our capacity for change, learn and gain new experiences and to learn self-actualization skills, i.e. our ability to silence our ego. How much we have silenced our ego, i.e. the level of our experience, is our current state of emotional maturity – awareness (Muk, 2007).

Maturity - awareness itself was mapped by David R. Hawkins in 2013. It corresponds almost completely to the concept proposed and described in this article, and certainly provides an insight into the levels of maturity- consciousness (Hawkins, 2022). However, this model does not show that maturity comes from balance, so it does not accurately visualize the truth. Additionally, it does not provide the full context and explanation of how we progress through the levels with self-actualization skills and emotional intelligence, and it incorrectly positions fear and courage.

In line with everything stated here, in Figure 2 there is a proposal for a new map, that is, the level of emotional maturity - awareness. It completely correlates with the pyramid of self-realization, but is described from the perspective of the meaning of life.

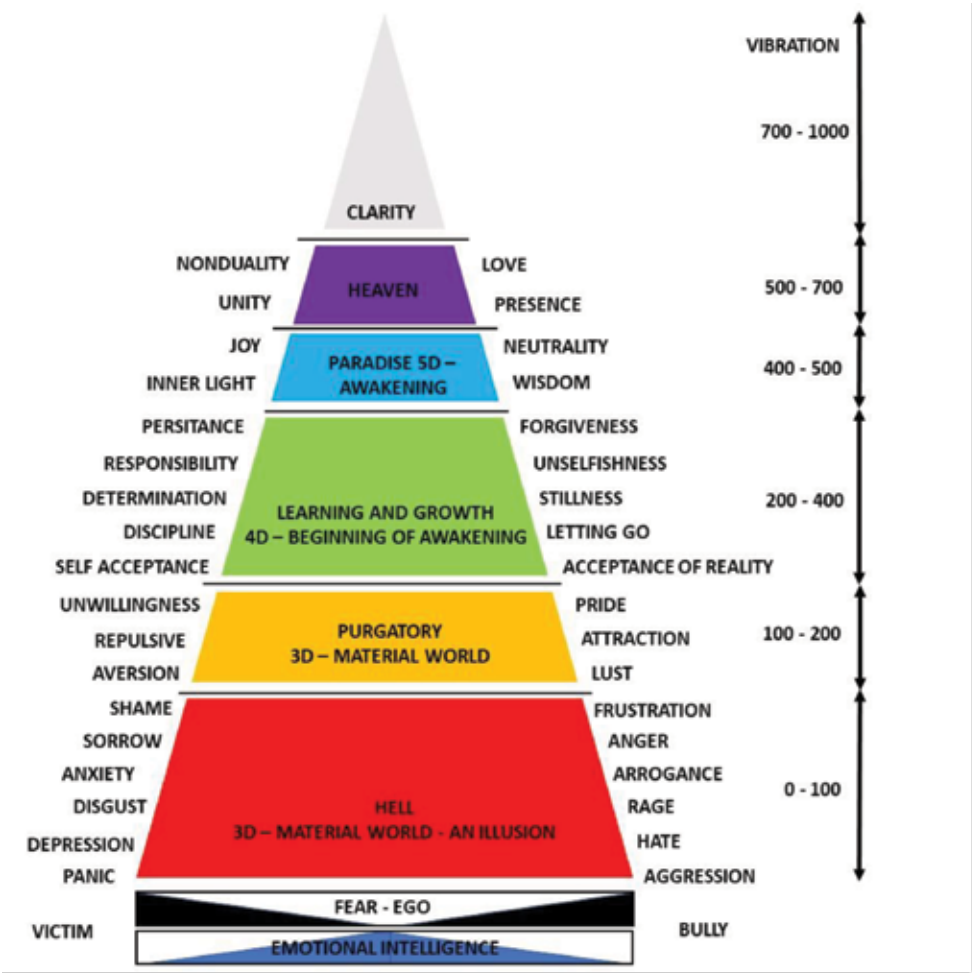

Figure 2. Levels of maturity - awareness

The first level of maturity is complete inexperience in which there is a complete rule of the ego and the absence of our emotional intelligence, our only tool for learning. This level is also called hell because it is full of daily emotional suffering and consequent problems.

At the next level, the ego is quieter, there is a certain shift in maturity due to the accumulated experience. Extremes are much rarer, satisfaction is greater, everyday problems are fewer and fewer, and relationships are getting better. Nevertheless, none of that has disappeared yet, we are still in the material, mental, world and see nothing but the outside world where we fulfill our needs, mature and further cleanse ourselves of our ignorance - ego, which is why this level is called purgatory.

After we have gathered enough experience, we begin to notice that the outside world does not provide us with answers to our questions, which we ask more and more often. We then start looking for the answers and *truth* in our inner world by changing ourselves and learning the skills of self-realization. It is the beginning of awakening from the illusion of the importance of the material world that we used to be in. This can be quite a confusing level because a person is waking up and everything that was important to him/her loses its importance, and everything the person sees begins to be seen with different perspective. However, it is necessary to understand that the beginning of awakening does not mean being awake. Although we begin to perceive the *truth*, it does not mean that we are living it yet: we have just become aware of it. At this level, we are expected to learn all the skills of self-realization and continue to create until the moment we wake up.

At the next level, nothing from the outside world can control us anymore and we achieve neutrality or indifference, but at the same time reach complete joy and wisdom. This level is characterized by the fact that it is not a process, but a precisely defined moment that is not accompanied by any audiovisual effects. Suddenly, we get a feeling of complete clarity. Every day after this, emotionaly, is like paradise for us. It is necessary to understand that even at this level we are still not completely devoid of emotional reactions. However, they no longer control us, and life itself becomes completely clear to us.

We begin to realize that we are all one, and with that realization we get a huge need to share the knowledge and clarity we have received, and thus realize love and enter heaven. The characteristic of this level is that we will not even know when it happened to us, because only once will we realize that our priorities, that is, our perspective and what is important to us, have drastically changed. Love is the ultimate skill, with the knowledge of which we move to a new level and realize that we are part of a bigger picture. With this, everything becomes unimportant to us except other people and their knowledge and needs. It is necessary to understand that we do not deny ourselves, but feel sincere satisfaction in helping others in any way, especially the one in which we ourselves have the most inclination and experience.

In the end, when we lose all emotional reactions and needs - since even the need for knowledge or giving and sharing is egoistic because it comes from inexperience - we gain complete maturity and clarity, and fully enter the present moment. However, paradoxically, as always, in order to mature and raise our own awareness, we must first enter our depth, our inner world (Brach, 2021; LaPera, 2021; Siegel, 2010), because we raise our maturity - awareness in the long term only by overcoming our conscious and unconscious fears.

Conclusion

In this paper, we presented and connected a number of new concepts of the emotional functioning of us humans. The purpose of this paper was to break a series of misconceptions related to the meaning of life, self-realization, ego and emotional intelligence, and our emotions. Further on, the goal of this paper is to concisely outline all the information related to our inner world in one place and connect all these parts into one broad picture so that knowledge can be passed on to future generations as simply as possible. The only question in the end is, is this paper the truth? If it is, then we need to start revising the textbooks we use for teaching our children.

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Overview of Variations in Liver Anatomy and Their Clinical Significance

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Abstract

The liver performs numerous vital functions in the human body, including detoxification, nutrient metabolism, and protein synthesis. Although it is typically divided into two main lobes, the right and left, the liver can also exhibit rare anatomical variations that may impact clinical outcomes.
These variations include agenesis of the right or left hepatic lobe, accessory hepatic sulci, and variations such as the "Beaver tail" liver, Riedel's lobe, pseudolipoma of Glisson's capsule, supradiaphragmatic liver, and ectopic liver tissue.
Modern imaging techniques, such as computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound (US), play a crucial role in identifying and characterizing these variants.
A thorough understanding of these anatomical variations is essential for accurate diagnosis, effective treatment planning, and preventing complications during surgical procedures.
A multidisciplinary approach and knowledge of liver anatomical variants can improve the quality of care for patients with liver diseases.

Keywords: Liver Anatomical Variants, Modern Imaging Techniques, Clinical Significance, Surgical Implications

Sažetak:

Pregled anatomskih varijanti jetre i njihova klinička važnost

Jetra obavlja niz vitalnih funkcija u ljudskom tijelu uključujući detoksifikaciju, metabolizam nutrijenata i sintezu proteina. Iako je tipično podijeljena u dva glavna režnja, desni i lijevi, moguće su i rijetke anatomske varijante koje mogu utjecati na kliničke ishode.
Ove varijante uključuju agenezu lijevog ili desnog režnja, akcesorne sulkuse,te varijacije poput "dabrovog repa", Riedelovog režnja, pseudolipoma Glissonove kapsule, supradijafragmalnu jetru i ektopično tkivo jetre.
Moderne slikovne tehnike poput kompjutorizirane tomografije (CT), magnetske rezonancije (MR) i ultrazvuka (US) igraju ključnu ulogu pri identifikaciji i karakterizaciji ovih varijanti.
Temeljito poznavanje I razumijevanje ovih varijanti važno je za postavljanje točne dijagnoze, učinkovito planiranje terapije te prevenciju komplikacija tijekom kirurških zahvata. Multidisciplinarni pristup i poznavanje anatomskih varijanti jetre može bitno pridonijeti kvaliteti skrbi za pacijente s bolestima jetre.

Ključne riječi: anatomske varijacije jetre, modern slikovne tehnike, klinička važnost, kirurške implikacije

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This article was submitted to RAD CASA - Medical Sciences as the review article

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 2 May 2025
Accepted: 29 May 2025
Published: 25 June 2025

Citation:
Coce N, Filipović-Grčić L, Režan R, Planinić M, Mandić Paulić T. Overview of Variations in Liver Anatomy and Their Clinical Significance

567=70-71 (2025): 60-63
DOI: 10.21857/y54jof4k3m

Introduction

As the largest solid organ in the human body, the liver plays a critical role in numerous vital processes, including nutrient metabolism, protein synthesis, detoxification, and glycogen storage. Despite its primary anatomical division into four lobes – the right hepatic lobe (lobus dexter), the left hepatic lobe (lobus sinister), the caudate lobe (lobus caudatus), and the quadrate lobe (lobulus quadratus) – as well as its eight functional units according to Couinaud classification, the liver can exhibit rare but significant morphological variations. These include the presence of additional lobes or sulci. Among anatomical variants, we distinguish accessory hepatic lobules such as agenesis of the right or left hepatic lobe, "Beaver tail" liver, Riedel's lobe, supradiaphragmatic liver, and ectopic liver tissue. Additionally, entities include pseudolipoma of Glisson's capsule and accessory hepatic sulci.(1)
Anatomical variations, particularly accessory liver lobes, can influence diagnostic and therapeutic procedures. (2) Radiological techniques such as CT and MRI play an important role in identifying these variations, providing crucial information that may affect treatment decisions, particularly in surgical interventions such as laparotomy for gallbladder surgery or liver transplantation. (3)
This review aims to describe various anatomical variations of the liver, examine their incidence, anatomical presentation, clinical significance, and how they may impact diagnostic outcomes. A better understanding of these variations will allow physicians to improve diagnostic and therapeutic procedures, ultimately enhancing patient care.

Agenesis of the Right or Left Hepatic Lobe

Agenesis of the right or left hepatic lobe is an extremely rare anatomical variant in which there is a congenital absence of either the right or left hepatic lobe, often accompanied by compensatory hypertrophy of the remaining liver segments.(4,5)
It is essential to distinguish hepatic lobe agenesis from atrophy or hypoplasia caused by disease or injury, as well as from surgical resections that lead to liver lobe reduction. Agenesis of a single hepatic lobe is usually asymptomatic, whereas agenesis of the entire liver is incompatible with life. (5)
Recognizing these anatomical variations is crucial when planning surgical interventions and interpreting imaging findings. (4,5)

Accessory Hepatic Sulci

Accessory hepatic sulci, also known as secondary or additional grooves on the liver surface, are indentations not typically present in standard liver anatomy. They can appear on any liver surface but are most commonly found on the diaphragmatic surface. Although usually asymptomatic, the presence of accessory sulci can mimic benign or malignant liver lesions during CT imaging studies. (6)

"Beaver Tail" Liver Variant

The "Beaver tail" liver variant (Figure 1) is a rare anatomical phenomenon in which the liver extends laterally and anteriorly, adopting a shape reminiscent of a beaver's tail. This variant is more commonly found in women. Although mostly asymptomatic, it is crucial to differentiate this variant from tumours, hepatomegaly, or other changes that may present with similar radiological findings. Moreover, the presence of this liver variant can complicate hepatic and abdominal surgeries, necessitating careful consideration during surgical planning. (2)

Figure 1: Beaver tail liver. An axial plane of a CT scan of the liver shows an anatomical variant of an accessory liver lobule where a portion of the parenchyma atypically extends laterally to the left and anteriorly, taking on a shape reminiscent of a beaver's tail.

Riedel's Lobe

Riedel's lobe (Figure 2) is an elongation of the right liver lobe extending below the lower rib margins down to the iliac crest. First described by Bernhard Moritz Carl Ludwig Riedel in the 19th century, this anatomical variant correlates with hypertrophy of liver segments V and VI. (2)
Previously believed to be more common in women, later studies found that its incidence is equal in both sexes. (7 ,8) While Riedel's lobe is typically asymptomatic, it can sometimes be mistaken for a tumour mass during palpation. Additionally, its presence can lead to complications during hepatic surgeries or result in torsion of the lobe or primary and secondary tumour formations.(9)

Figure 2: Riedel's lobe. A coronal CT plane shows an elongation of the right side of the liver extending below the lower rib ends down to the iliac bone, forming an anatomical variant of an accessory liver lobule known as Riedel's lobe.

Figure 3: Pseudolipoma of Glisson's capsule. An axial CT plane shows a cluster of normal fatty tissue (arrow) located around the liver within Glisson's capsule, thus forming a distinct entity in the anatomical variation of the liver - Pseudolipoma of Glisson's capsule.

Figure 4: Supradiaphragmatic liver. Coronal CT plane shows an anatomical variant of the liver where a portion of the liver tissue is located above the diaphragm in the thoracic cavity, also known as the intrathoracic accessory liver.

Pseudolipoma Of Glisson's Capsule

Glisson's capsule is a thin layer of connective tissue surrounding the liver. A pseudolipoma refers to the presence of fatty tissue resembling a lipoma but consisting of normal fat tissue located within or around the liver, particularly within Glisson's capsule (Figure 3). (10) First described by Rolleston in 1891, pseudolipomas of Glisson's capsule are generally asymptomatic and are often discovered incidentally during imaging studies, intraoperatively, or during autopsy. In most cases, pseudolipomas do not require treatment unless they cause mechanical issues or complicate the diagnostic process by resembling other potentially malignant fatty tissue tumours. (11)

Supradiaphragmatic Liver

Supradiaphragmatic liver (Figure 4) is a rare anatomical variant where a portion of the liver tissue extends above the diaphragm, sometimes referred to as "intrathoracic accessory liver." The size of the ectopic liver tissue can vary from a small mass to a significant mass occupying space in the thoracic cavity, where it may be mistaken for pulmonary or pleural tumours. Clinically, this variant can impact respiratory function, particularly if the hepatic tissue volume is substantial. Understanding this variation helps avoid potential surgical complications and allows for more accurate treatment planning. (12)

Ectopic Liver Tissue

Ectopic liver tissue, also known as liver heterotopia, is a rare anatomical variant characterized by the presence of liver tissue outside its usual anatomical location. This tissue can be found in various locations, including the gallbladder, abdominal cavity, or other organs such as the stomach and kidneys. It is crucial to differentiate ectopic liver tissue from primary tumours or metastases. In some cases, ectopic liver tissue has been found to be the site of hepatocellular carcinoma, indicating an increased risk of hepatocarcinogenesis. (13)

Conclusion

Liver anatomical variations, while often asymptomatic, can significantly impact diagnostic and therapeutic outcomes. Understanding these variations is essential for accurate diagnosis and surgical planning. Radiological imaging techniques such as CT, MRI, and US are invaluable tools in detecting these variants, enabling better preparation and adaptation of diagnostic and therapeutic procedures. This review serves as an educational resource for physicians, emphasizing the importance of recognizing liver anatomical variations.

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Map of Humans Inner World

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This article was submitted to RAD CASA - Medical Sciences as the review article

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 23 April 2025
Accepted: 29 May 2025
Published: 25 June 2025

Citation:
Salopek Z, Demarin V. Map of Humans Inner World

567=70-71 (2025): 64-74
DOI: 10.21857/mnlqgc35py

Abstract

This paper presents a new model, rather a map of our inner world. Through the *truth*, the paper will explore how our internal motivation shapes our experiences and, consequently, our level of maturity. Furthermore, the paper explores and describes the specific *ego challenges* that we all face on the path to self-actualization, fueled by fears that unconsciously stear our life. More importantly, this paper examines the self-actualization skills that ego and our *ego challenges* are meant to teach us. In conclusion, it emphasizes the importance of emotional intelligence and proposes new model as a solution for overcoming ego and contributing to emotional maturation or raising one's awareness.

Keywords: *Truth. Ego. Emotional intelligence, Fears*

Sažetak:

Karta unutarnjeg svijeta čovjeka

Ovaj rad predstavlja novi model, odnosno kartu našeg unutarnjeg svijeta. Kroz istinu, rad će istražiti kako naša unutarnja motivacija oblikuje naša iskustva i, posljedično, našu razinu zrelosti. Nadalje, rad istražuje i opisuje specifične izazove ega s kojima se svi suočavamo na putu samoostvarenja, potaknute strahovima koji nesvjesno upravljaju našim životom. Što je još važnije, ovaj rad ispituje vještine samoostvarenja koje nas ego i naši izazovi ega trebaju naučiti. Zaključno, naglašava važnost emocionalne inteligencije i predlaže novi model kao rješenje za prevladavanje ega i doprinos emocionalnom sazrijevanju ili podizanju nečije svijesti.

Ključne riječi: Istina. Ego. Emocionalna inteligencija, Strahovi

Inner world map

All that we are and how we think and feel today, how we behave and all our emotions and the consequent words and actions are again only a consequence of our level of maturity, that is, experience. To understand our thoughts, emotions and behavior, it is important to understand our inner world. It starts with what is seen, our personality and our emotions.

Emotions are an inseparable part of human personality. Or are they not? On the one hand, all our or other people's bad decisions, all the mistakes we made or that were made to us, all the pain we experienced or caused to others came from our or other people's emotions. Emotions are like mud in a clear glass of water. When the glass and the water are clean, everything we see, we see it as it really is. We see ourselves, the people around us and the events in a true and clear way. When emotions gets our water dirty and turbulent, everything we see through the murky water of emotions, positive and negative, is wrong and distorted (Holiday, 2021; Winnicott, 1971). Even though everything is distorted and we cannot see anything clearly, we do not know it until the emotion passes. Due to our emotions, we have not seen reality as it really is, but have created an image of how it could be, how it was, how we think it should be, and we do not see the "naked" and "cold" *truth* that would solve all of the above problems caused by emotionality. People often refer to their own emotionality as an advantage without realizing that it is the manifestation and solution of the problem (Pervin, Cervone, & John, 2008).

On the other hand, because of love and understanding, commitment and acceptance, people are ready to face mortal fears. They are ready to sacrifice their whole lives because of their passion, and even give their lives away for their children. Due to a sincere feeling of gratitude, they are ready to devote their whole lives to complete strangers or give away all their possessions, and because of love, people are ready to make superhuman efforts (Colman, 2003).

Due to the lack of clarity, all human reactions are pushed into the same basket in the existing models of emotions (Plutchik's model of emotions as example). Thus, anger and stubbornness, resentment, acceptance and gratitude all end up there. Nevertheless, that is not the *truth*.

It is true that anger is an emotion that we physically feel and as such is a consequence because it is always triggered by an external stimulus and has a limited lifespan. It is there only to warn us that we have deviated from the *truth* and that our current perception is not correct, and to start looking for the cause of our anger. It is also the *truth* that stubbornness is not an emotion, but rather our emotional state resulting from our current level of maturity. It is mental and more permanent, but changing that state is the very meaning of life. Resentment is an *ego challenge* and the cause of our stubbornness and anger. Acceptance and forgiveness are skills that we must learn by studying them in order to stop holding grudges and thus stubbornness and anger will automatically disappear just as any effect disappears by solving the cause. Gratitude is state, not an emotion, that we receive as a reward when we learn acceptance and forgiveness, and thereby remove our resentments (Mehmood – Shaukat, 2014).

It is crucial to remember that we cannot influence our personality and level of maturity, at least cognitively and to a greater extent, directly, but only by silencing the ego, i.e. facing our fears, and personality and level of maturity will develop as a result of overcoming these fears and learning skills of self-realization. So we cannot train gratitude, joy or stress resistance, but we need to face our fears and learn acceptance, forgiveness and similar skills. Gratitude, joy or emotional stress resistance will come as a reward for the fears we have overcome. By the same logic, we cannot influence much the way our personal and business success will unfold, but we can mature and success will happen as a consequence of our maturing.

In order to completely categorize every human reaction as a consequence or cause of our maturity, in Figure 3 one can find a detailed map of our inner world with a diagram of emotions, clearly listed *state of ego* and *state of love* (personality), and *ego challenges* that are the causes of our personality and emotions and the skills we need to learn. Additionally, the fears that are the causes of all our *ego challenges* and emotional intelligence as a solution for overcoming our fears are clearly listed (Kardas et al., 2019).

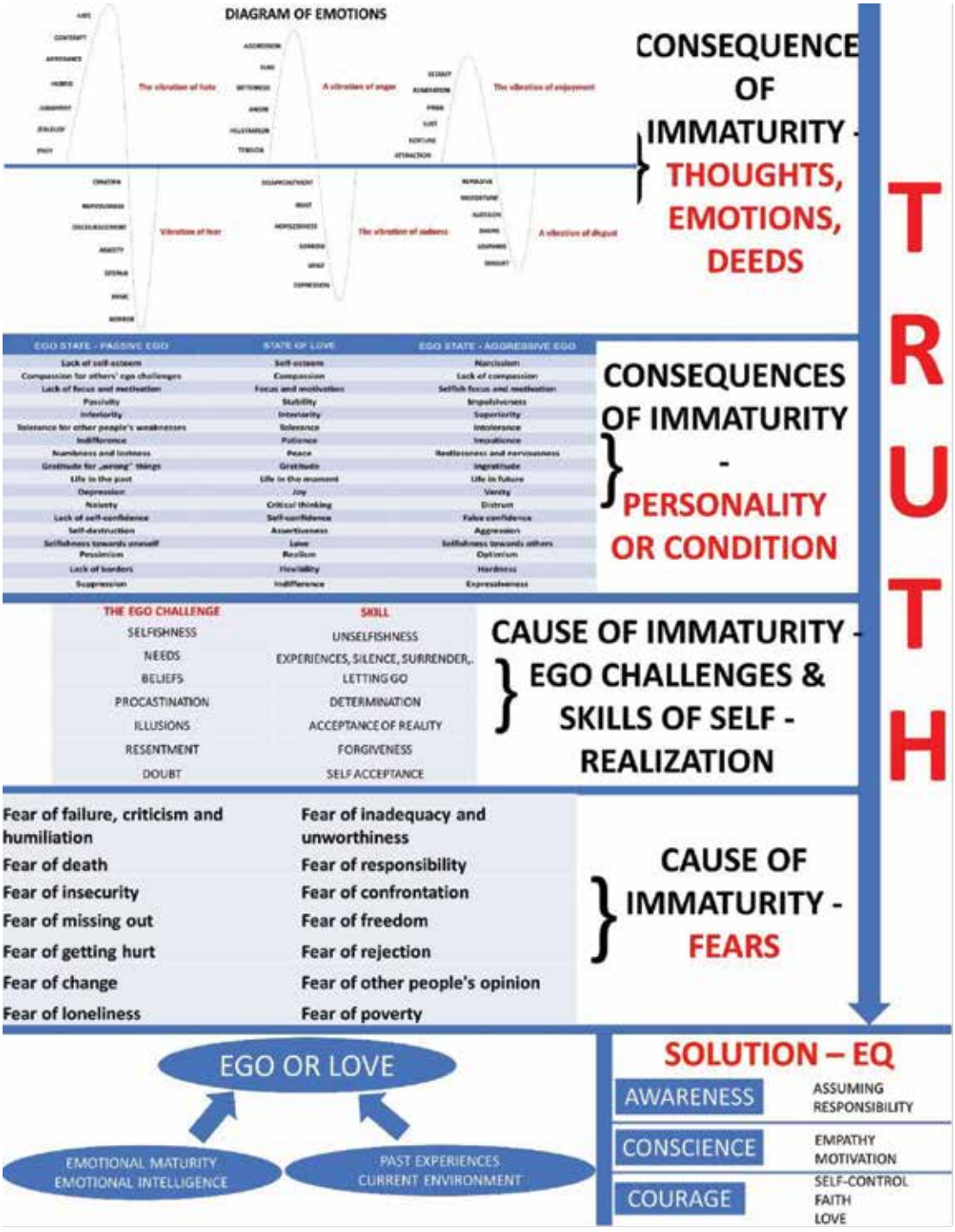


Figure 3. Map of the inner world

The map outlines our inner world; for example, we are stressed because we constantly expect a lot from our family or life. We have expectations because we have a strong need for control due to the fear of the unknown. So the *ego challenge* is to face its need for control and learn the skill of letting go. It is up to us to face our expectations, not the resulting anger and what it causes in our business and personal lives. This means that when we learn the skill we need (letting go) and thereby remove the cause (the need for control and consequent expectations), the consequences (stress and problems at work) will disappear by themselves. That is how we get to know ourselves and mature, raise our own awareness. Therefore, the goal of self-actualization is emotional maturation by learning the skills that will allow us to understand the skill of love, and this is possible only if we follow the *truth*.

Truth

By following the *truth*, we gather experiences and consequently mature. Wise men have always said the same thing. Jesus said: "Know the *truth* and it will set you free." Socrates declared: "We follow the *truth* wherever it leads us." Buddha said: "Follow the path of *truth*. Think about it. Make it yours. Live it. It will always take care of you." The great psychoanalyst Wilfred Bion said a long time ago that the whole of psychoanalysis and psychotherapy is nothing but a great search for the *truth* (Bion, 1962). It is only with it that it is possible to achieve true recovery. Nevertheless, the problem is that the message can be lost if it is misinterpreted. So what is really the *truth*?

The simple *truth* may be that we are really not good enough for a job, or maybe we have outgrown our environment and need to leave. It can also be the *truth* that in our insecurity we are often angry and frustrated or aggressive and intrusive. The *truth* may also be the fact that we have no consciousness or conscience. Maybe even no courage. It may be that we criticize each other a lot, and are unable to forgive each other. The *truth* is also that we often do not manage our own lives. If we are arrogant, the *truth* is that we behave that way because we want to be better than others out of our own insecurity.

Truth requires a deep dive into ourselves to discover what genuinely motivates and drives us. For example, if, due to fear of loneliness, we remain in a bad relationship, let us not be surprised by the misunderstanding and dissatisfaction we feel. The *truth* is that the fear of being alone causes us to suffer. If we are not living the life we want because of fear of change, let us not be surprised by the depression we feel. Although this is often unacceptable to us, the *truth* is that in the outside world the only constant is change. Attachment to the current state or fear of stepping into the unknown only brings suffering. The *truth* is also that our fear of taking responsibility can be our biggest challenge right now.

The *truth* is also that we are quite disconnected from ourselves and our own potential if we have accepted the belief that material things or beliefs are the way we will prove our worth. The *truth* is also that selfishness towards others is a weakness, just like selfishness towards oneself. The *truth* is that doubt is the biggest inhibitor of personal growth, but also that we are the only ones who can silence it.

The *truth* can also be the fact that something is simply not important. The *truth* is objective, honest and benevolent for all who can accept it. It exists beyond our emotional reactions to our own fears. It is real, regardless of how we perceive it. It leads us out of unconsciousness and introduces us to a life of complete maturity (Jung, 1998). The *truth* is the way.

There are many *truths*, and we often do not like them. We thus look in the other direction as if the *truth* will go away. It never leaves. No matter how much we ignore it, sooner or later it appears. If we continue to ignore it, the manifestations are getting worse, and the lessons to overcome the challenges and accept the *truth* are getting harder and harder. We are in a school called life and we will not pass until we have solved all the lessons. The excuse that we did not see the *truth* and did not understand it is not accepted. We are expected to look for it and gather experiences based on it.

We can rationally understand the *truth*, like everything else, as an insight at a conscious, external level or truly and deeply know it at an internal level. The realization is an A-ha! moment or epiphany, that is, a unique or indescribably deep insight related to our life or the patterns that are repeated in it preventing us from living our best life. When we rationally understand that it is not good to procrastinate, and we still behave like that, we say that we have rationally understood it on a conscious level, but we cannot yet live it. When we practice determination long enough and experience the aforementioned A-ha! moment or epiphany and we honestly and deeply understand why it is important to be determined and really start to live that determination without thinking about it, then we can say that we have grasped the experience of determination.

The most unpleasant *truth* for us humans is that we do not know. Out of ignorance, our ego does not allow us such a simple and at the same time comprehensive knowledge of ourselves. The *truth* that we do not know anything about viruses and vaccines, that we do not know anything about the person we judge and avoid, that we do not know ourselves and our fears, that we do not know if something is luck or misfortune. We are not aware that we do not know. When we do not know the *truth* and form prejudices and opinions based on "feelings", we are actually listening to the ego screaming in our ear. It is therefore better not to have an opinion, because the *truth* is that we do not know the *truth*. This is so because thinking makes it impossible to know the *truth*. By attaching ourselves to our own opinion, we prevent learning and maturing. The ignoring of the *truth* and the resulting immaturity leads to emotions.

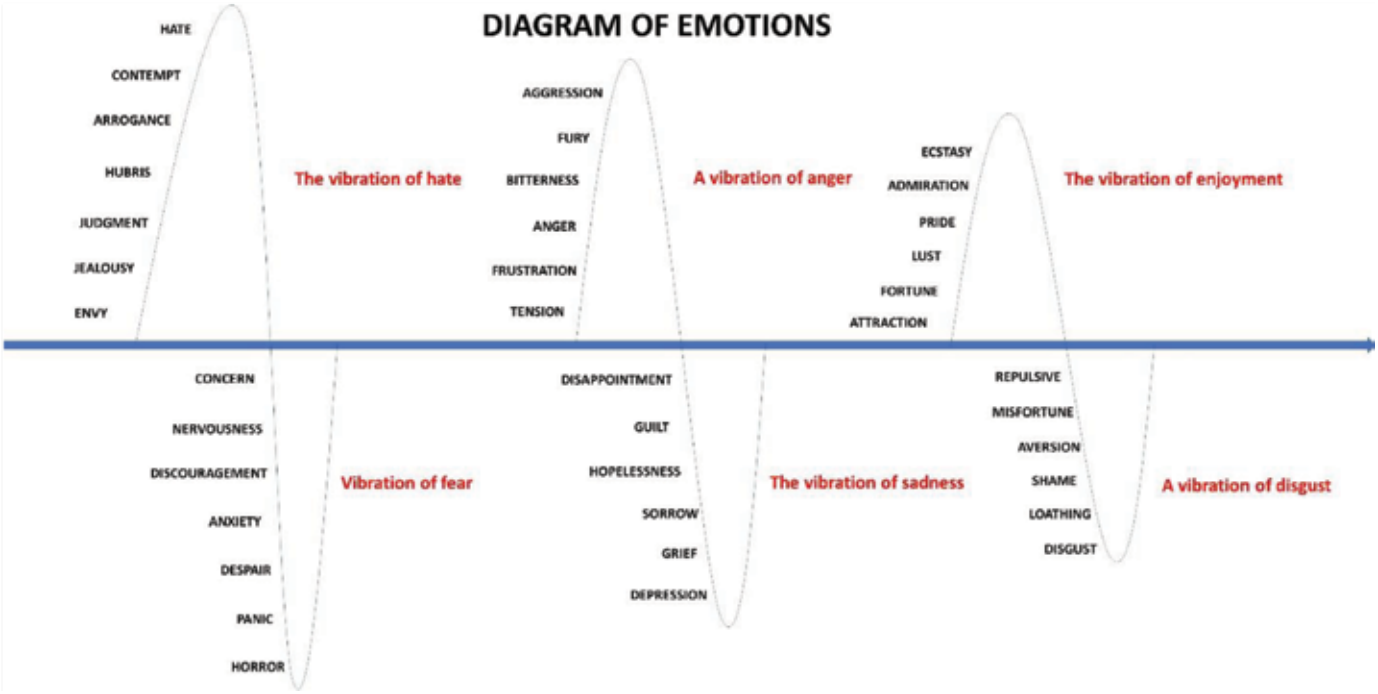


Figure 4. Diagram of emotions

The consequences of our immaturity; our thoughts, emotions and actions

Find the emotion diagram in Figure 4. The characteristic of this level is volatility in accordance with the natural law "as up, so down", which says that everything that goes up must go down. Like our emotions, so our life. The strength of hatred corresponds to the strength of fear in the person, the strength of anger will correspond to the strength of grief that will follow, and the strength of adoration will correspond to the strength of disgust towards something else. According to the natural law, "as within, so outside", in our external world, the strength of our greed will correspond to the depth of business or private problems that we will fall into as a result. The more immature and ego-driven a person is, both he/she and his/her life will be more volatile with greater amplitudes. The more experienced and mature a person is, the more stable both the person and his/her life will be (Salopek, 2023).

Here it is necessary to understand the *truth* that the only meaning of emotions is to make us aware, as an alarm warning us that we have a wrong perception of reality and that we are governed by ego (ignorance and fear), and not love (strength and knowledge). Emotions are a guide to our triggers, to our challenges and fears (Kiriaku, 2001).

The *truth* is that all emotions arise from our ignorance - ego weaknesses. However, this does not mean that they are "wrong", the *truth* is that they are necessary and therefore welcome because they are a signpost to our ego, our weaknesses, inexperience and trauma. However, with that logic, when there is no weakness - there are no emotions. Further on, when there are no emotions - we feel great, calm, fulfilled, we can truly love. Here it is necessary to understand that emotions and feelings are not the same. Emotions are physical and feelings are mental (I am sad and that is why I feel bad, I am not sad and that is why I feel great) (Mehmood – Shaukat, 2014).

It is also the *truth* that our inner world always manifests itself in our outer world. Thus, due to envy and jealousy we destroy relationships, due to anger and rage jobs fail, due to sadness and unfulfillment people get psychosomatic diseases, due to fears everything that we are afraid of losing runs away from us. Our health, our private and business life, our external world, as well as our emotional world, is only a consequence of our maturity, that is, our experience. The more experience we have, and the more things are unimportant to us, the less weakness and immaturity we have. The less weakness we have, the fewer emotions we have. When we accept reality with full clarity, then we do

not get involved in the emotional evaluation of that same reality, so it can be called neutrality or it can also be called complete inner peace that everyone is looking for so much, depending on the maturity of the one who interprets. Therefore, in order to understand exactly what it means to be emotional, we must first understand that there are no "positive" and "negative" emotions (Salopek, 2023).

All emotions are just an alarm warning our on our ignorance - ego. How is this possible and how to recognize them? In fact, very simply, just let us not misinterpret the "positive" emotions caused by ego satisfaction with a *state of love*, joy or gratitude. There are two simple ways to learn to tell them apart (Nvankvo, 2019).

Firstly, the satisfaction of the ego is always extrinsic, caused by some entity or idea from the outside world, and the *state of love* is intrinsic and comes from within us, from our inner world. Sincere feelings of joy, happiness, gratitude and love are not conditioned by external factors but solely by internal maturity. We can only be truly grateful for reality, whatever it is, and gratitude does not depend on external influence. When we overcome and silence the ego by overcoming the *ego challenges*, joy and gratitude flow from the existence itself. They come from within us without any external conditioning, and it is from this source that we can see the difference between ego satisfaction and the *state of love*. (Chartrand – Bargh, 2002).

Secondly, all emotions, both positive and negative, no matter how intense they are, are always fleeting. When we reach the *state of love*, when we mature emotionally, that state is no longer fleeting. Joy and love are always present because they do not depend on anything.

For example, fi we buy a car, because we want to prove ourselves, and not out of necessity, that purchase will cause us joy, happiness and gratitude, at least we think these emotions are called that. However, the *truth* is that it means satisfying our ego. That satisfaction of the ego will tie us to a certain thing or idea. It will make us chase that feeling of happiness again, which we felt the first time when our expectations were fulfilled. Thus, we will move away from the *truth* and the true joy and gratitude, our potential and our purpose, because we will focus on, in this case, cars, and think that they will bring us happiness. Are those emotions that we interpret as happiness, joy and gratitude positive? They will last for a while, shorter in some, and in others longer, but they will definitely be temporary until we want something else. In case that desired car is stolen from us, there will be anger and rage, then grief and sadness (Kiriaku, 2001).

Both positive and negative emotions were fleeting and caused by an external factor, the car in this case. It is as if we have given the remote control to some entity or idea to control how we feel. If the external factor is favorable, we are happy, and if it is unfavorable, we are unhappy. Both are the path of the ego, because neither up or down, praise nor criticism should motivate us (Salopek, 2023).

We do not want to give our environment that much power to control us. That is why emotions are just a guide serving to help us, through self-examination, to get to our character trait that triggers our emotions and all the consequences that come with it. (Chartrand – Bargh, 2002).

Consequences of immaturity - personality

The inner manifestation of the ego in a person or ourselves is actually our state, our current level of maturity, that is, our experience or lack of experience. In fact, it is our personality. Although personality is as much a consequence of our maturity as emotions, and although it is changeable, it is still more stable than emotions. It is that part of us when we say "I am like that". The problem arises when we really think that we are like that and unchangeable, and new experience and change in everything, including our personality, is the very meaning of life. In Table 1, there is a list of all the consequences of our maturity expressed through personality and its extremes (Ruthig et al., 2007).

Table 1. Balanced personality (state of love) and its extremes (state of passive and aggressive ego)

EGO STATE - PASSIVE EGO	STATE OF LOVE	EGO STATE - AGGRESSIVE EGO
Lack of self-esteem	Self-esteem	Narcissism
Compassion for others' ego challenges	Compassion	Lack of compassion
Lack of focus and motivation	Focus and motivation	Selfish focus and motivation
Passivity	Stability	Impulsiveness
Inferiority	Interiority	Superiority
Tolerance for other people's weaknesses	Tolerance	Intolerance
Indifference	Patience	Impatience
Numbness and lostness	Peace	Restlessness and nervousness
Gratitude for „wrong" things	Gratitude	Ingratitude
Life in the past	Life in the moment	Life in future
Depression	Joy	Vanity
Naivety	Critical thinking	Distrust
Lack of self-confidence	Self-confidence	False confidence
Self-destruction	Assertiveness	Aggression
Selfishness towards oneself	Love	Selfishness towards others
Pessimism	Realism	Optimism
Lack of borders	Flexibility	Hardness
Suppression	Indifference	Expressiveness

Everything we think, say and do and how we live is a consequence either of ego or love. The ego speaks with fear and manifests the state of the ego (for example, inferiority or superiority), and love responds to the same by manifesting the state of love (interiority). Both are consequences, positive or negative, of following or not following the *truth*. Sometimes it seems that the manifestations, that is, our personality, whatever it is, are given and unchangeable, and that is why we all say: "I am that kind of person." We need to know that we are not "that way", that it is not the *truth*. What we currently are is only a reflection of our experiences (Chartrand - Bargh, 2002). We can only understand so much, nothing more and nothing less, because that is our current maturity and limit of understanding. We need to overcome a lot of challenges to find out who we really are, starting with the innate fear of change, and set out on the path of further maturation, because determination as such does not exist in maturation. For example, if an introvert faces his/her own fears and overcomes them, he/she will no longer be an introvert. If an extrovert suppresses his/her need to stand out, he/she will no longer be an extrovert. It is only through emotional maturation and the lessons hidden in *ego challenges* that we truly discover who we are (Petri & Govern, 2012).

The cause of immaturity - *ego challenges*

Ego challenges are a mirror of our inner world. Thus, if we become aware that we often procrastinate the performance of tasks that we consider challenging, through self-examination we will come to the motive (Buggle, 2007; Piaget, 1950), in this case that we may be procrastinating due to the fear of failure. Further on, if we realize that the way we look is very important to us because we are tied to the idea of physical beauty, through self-examination we can realize that we are afraid of other people's opinions. Additionally, if we become aware that we tend to get into conflicts all our lives, maybe through self-examination we realize that the cause is our selfishness (Pervin, Cervone, & John, 2008).

It is necessary to understand that all *ego challenges* are not here to harm us, but only to provide us with a challenge and a training ground for learning the skills of self-realization, with the realization of which they disappear. Thus, selfishness wants to teach us selflessness, procrastination determination, illusions and doubts to accept the reality and ourselves, and our beliefs want to teach us the skill of letting go.

Below in Table 2, all *ego challenges* and skills that we need to learn by overcoming these challenges are listed, and the balance in which we will find these skills (Salopek, 2023).

Table 2. Ego challenges, self-realization skills and the balance in which they are found

EGO CHALLENGE	SKILL	BALANCE
Selfishness	Unselfishness	Taking care of ourselves, but also of others
Needs of the outside world	Experiences	Fulfilling of our needs, but not at the expense of others
Need for control	Surrender	Surrendering to life, but being mindful in discovering the Path we need to walk
Need for opinion	Stillness	Being quiet and admitting that we do not know, but speaking when we learn and when they ask
The need to create	Persistence	Not trying, but being committed and persistent, without expecting an outcome
The need for socialization	Understanding	Learning to understand, but accepting that we will not always be understood
The need for comfort	Growth	Experiencing pleasure, but realizing that growth is outside of that same pleasure
Beliefs	Letting go	Letting go of all our beliefs, but only in order to truly understand who we are
Procrastination	Determination	Being determined, but choosing our battles
Illusions	Accepting reality	Accepting reality, but acting
Resentment	Forgiveness	Forgiving, but not forgetting
Doubt	Self-acceptance	Accepting ourselves, but alowing us to change

Behind all our *ego challenges* there are always fears.

The cause of immaturity - fears

Fears are exactly what makes us immature and inexperienced. Fears are what prevents us from progressing and overcoming of fears is exactly what will allow us to acquire new skills. This is so because fear, like all challenges, disappears only by facing that same fear (Pervin, Cervone, & John, 2008). We must go through it, get to know it, and only then does it disappear. Thus, if we really want to mature, we must gather courage, go through and experience insecurity, loneliness, failure, uncertainty, change and a hundred other experiences that cause us discomfort. A list of all conscious and unconscious fears is provided in Table 3.

Table 3. List of conscious and unconscious fears

Fear of failure, criticism and humiliation	Fear of inadequacy and unworthiness
Fear of death	Fear of responsibility
Fear of insecurity	Fear of confrontation
Fear of missing out	Fear of freedom
Fear of getting hurt	Fear of rejection
Fear of change	Fear of other people's opinion
Fear of loneliness	Fear of poverty

As always, dedication is expected in studying these fears and experience. This way failure and change and loneliness will become simply unimportant. The fear of missing out will turn into the joy of missing out. When there is no fear and all of the above is unimportant to us, we will attract all of the above into our lives simply because fear is exactly what keeps them away from our lives. If we are afraid of being alone, the aggressive ego will fight so as not to be alone, and the passive ego will retreat, making all possible concessions. If we are afraid of other people's opinion, the aggressive ego will assert itself to show that it is the best, and the passive ego will please those whose opinion it fears.

But the *truth* is that fear, like everything, has its place and must be in balance. Fear is the reason why we look around when crossing the road, fear is why we look into the sea before jumping. Fear is here not only to challenge us to mature, but also to protect us from real dangers from the outside world (Apter, 2001) .

In existing theories, fears and *ego challenges* are always declared to be the consequences of trauma. As can be seen in the map of our inner world, this is indeed true, but not complete. If we grew up in an "ideal" environment, that does not mean we will not have our needs, challenges and fears. Many of our challenges will be the result of pure inexperience unrelated to any trauma. The only way to gather experience is to use our only anti-ego tool - our emotional intelligence (Salopek, 2023).

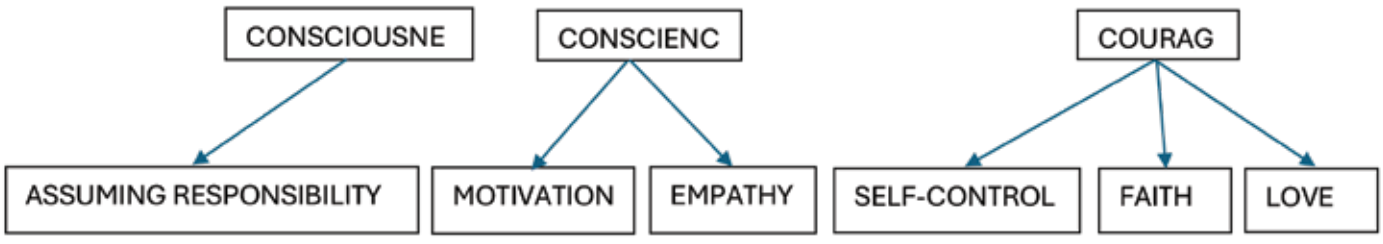
The solution to immaturity - emotional intelligence

Emotional intelligence is responsible for our inner world and it does not include intellect in the sense of technical knowledge and reading, but rather the ability to recognize, understand and manage one's emotions (Salovey, Mayer, & Brackett, 2007; Goleman, 1995; Bar-On, 2005). At least that is what the official definition says. In reality, in this world of duality, it is the only tool given to us as a counterweight to our ego; if ego is our emotional ignorance, our emotional intelligence is our ability to emotionally adapt, learn new skills. As we explained in our inner world map where we clearly defined what emotions, personality, skills and *ego challenges* are, our ego is the sole cause of our emotions that are actually alarm of our emotional ignorance - ego.

Therefore, emotional intelligence is actually our ability to silence our ego and consequently our emotions by overcoming our own needs and fears, and learning new skills. It tells us about our capacity for change and gathering new experiences, that is, about our capacity for the speed of maturing and raising our own level of maturity (Gruden, 1990).

Existing theories do not take into account the most important skills of an emotionally mature person, such as conscience or courage, and that is why we propose a new model in the broader sense of the meaning of life and self-realization (Figure 5).

Figure 5. Model of emotional intelligence



Our emotional intelligence, which consists of consciousness, conscience and courage, is actually the only tool for learning and solving *ego challenges* and the fears that fuel them and stand in the way of maturation. It is necessary in order to recognize other people's ego and our own (consciousness - self-consciousness), decide to act moraly and in accordance with the *truth* (conscience) and actually put it into action (courage). It also helps us to resolve the traumas hidden behind the fears on our way of realizing inner experiences. An unconscious person will say, "No, I do not know that I am doing something wrong. I am not aware of that." A person without a conscience will conclude: "I know I am doing wrong, but I do not really care." A less courageous person will hesitantly say: "I know what I am doing and I know it is wrong, but it is too hard for me to change." It is important to note that the developing of emotional intelligence is a challenge in itself, so first we need to develop consciousness, conscience and courage as tools to overcome fears and *ego challenges* and reach emotional maturity - awareness. Just like our ego, our emotional intelligence wants to teach us certain skills, given in Table 4 (Salopek, 2023).

Conclusion

In this paper, we presented and connected a number of new concepts of the emotional functioning of us humans. The purpose of this paper was to break a series of misconceptions related to the meaning of life, self-realization, ego and emotional intelligence, and our emotions. Further on, the goal of this paper is to concisely outline all the information related to our inner world in one place and connect all these parts into one broad picture so that knowledge can be passed on to future generations as simply as possible. The only question in the end is, is this paper the truth? If it is, then we need to start revising the textbooks we use for teaching our children.

COMPONENT	SKILL	BALANCE
Awareness	Responsibility	Let us take responsibility, but let others take responsibility for themselves
Conscience	Motivation	It does not matter what we do, it only matters why we do it
Courage	Discipline	Let us have discipline, but be flexible

Table 4. Components of emotional intelligence, self-realization skills and the balance in which they are found

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One New Hypothesis about the Ageing Process of Man

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Abstract

Ageing, the natural, complex, purposeful, polysyllabic and inevitable process in the life cycle of living beings, therefore also of man, according to today's knowledge, most likely takes place on the basis of a program located in the genome. This does not reduce the importance of dividing this process into normal or physiological aging and accelerated or pathological aging related to diseases. Life is essentially a conglomeration of more or less complex physiological and biochemical processes that are constantly taking place, and whose alteration or shutdown leads to its termination. An accompanying and inevitable phenomenon related to a large part of these processes is the generation of the so-called reactive oxygen species (ROS), molecular structures that are characterized by two crucial properties, aggression and destruction of molecular body structures, and signaling effects, as secondary messengers, on the mentioned processes. Among these, the oxidative destruction of exposed molecular structures, according to some of the latest findings, have special importance for the process of programmed aging. Among these structures, two large transmembrane multiligand receptors, low-density lipoprotein receptor-related protein 1 (LRP1) and receptor for advanced glycation end products (RAGE), and three enzymes deoxyribonucleic acid cytosine methyltransferases (DNMTs), and two Sp1 and Sp3 transcription factors (Sp1 and Sp3 proteins) otherwise abundantly present in tissues and cells, are specially affected. ROS elements lead to a strong activation, transcription and expression of those two factors, Sp1 and Sp3, resulting in their strong effect on DNMTs promoters with pronounced transcription and formation of DNMTs proteins. What is the function of DNMTs proteins? As components of crucial importance in the systems of epigenetics, these proteins condition the methylation processes of DNA molecules (adding the methyl group -CH3 to the molecules), generate the formation of 5-methyl cytosine (5mC) on the template strands of DNA, and the decrease in the transcription of methylated genes while shutting down their expression. Due to the specific conditions related to LRP1 and RAGE receptors, their promoters have different reactions to DNMTs-induced methylation. In LRP1 promoter methylation is fast and intense, while in RAGE promoter methylation is extremely slowed down and reduced. Thus, the final effects of those two genes, or their receptors, are extremely different. Biochemical and physiological processes related to LRP1 gradually slow down and dampen, and processes related to RAGE become more and more expressive. Another group of processes related to epigenetics and programmed aging includes oxidative demethylation of 5mC DNA segments via ten-eleven translocases (TET), thymine DNA glycosylase (TDG) and base excision repair enzymes (BER). Everything indicates that this second group of events is less efficient than the first group, and methylation clearly dominates. If nature hadn't programmed it that way, the aging process would be unpredictable. The increasing penetration of thought analyzes into the essence of the aging process, and the experimental results, increasingly point to the crucial importance of the process of transcription of the genes shown earlier. Regenerating transcriptions under the strong control of Sp proteins, and their programs located in the genome, influence the maximum possible life span of individuals of a species. The aim of this study is to provide additional explanations of the role of the mentioned receptors in the programmed aging of living beings. An additional goal of this study is the presentation of the latest findings on the

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This article was submitted to RAD CASA - Medical Sciences as the review article

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 27 April 2025
Accepted: 29 May 2025
Published: 25 June 2025

Citation:
Barić N. One new hypothesis about the ageing process of man

567=70-71 (2025): 76-91
DOI: 10.21857/ygjwrc2d2y

specific blockade of, in old age, increased expression of the RAGE receptor, and on the targeted gene therapy, also in old age, of the muted expression of the LRP1 receptor.

Keywords: Aging process, epigenetics, methylation and demethylation, LRP1 and RAGE receptors

Sažetak

Jedna nova hipoteza o procesu starenja čovjeka

Proces starenja, u biti prirodan, složen, svrsishodan, višeprofilan, i neminovan proces u životnom ciklusu živih bića, prema tome i čovjeka, po današnjim saznanjima, najvjerojatnije se bazira na programu lociranom u genomu. To ne umanjuje važnost podjele tog procesa u normalno ili fiziološko starenje, i ubrzano ili patološko starenje vezano uz bolesti.

Život je u biti konglomerat više ili manje složenih fizioloških i biokemijskih procesa koji se permanentno odvijaju, i čija alteracija ili gašenje dovodi do njegovog prestanka. Popratna i neminovna pojava vezana uz veliki dio tih procesa je generiranje tzv. reaktivnih metabolita kisika (ROS), molekularnih struktura koje se odlikuju sa dva krucijalna svojstva, agresijom i destrukcijom molekularnih tjelesnih struktura, te signalnim učincima, kao secundar messangers, na spomenute procese. Među tim, oksidativnom destrukcijom izloženih molekularnih struktura, po nekim najnovijim saznanjima, posebnu važnost za proces programiranog starenja, imaju dva velika transmembranska multiligandska receptora, low-density lipoprotein receptor-related protein 1 (LRP1) i receptor for advanced glycation end products (RAGE), te tri enzymes deoxyribonucleic acid citosine methyltransferases (DNMTs), i dva Sp1 i Sp3 transkripciona faktora (Sp1 and Sp3 proteins) inače obilno prisutna u tkivima i stanicama. ROS elementi dovode do snažne aktivacije, transkripcije i ekspresije ta dva faktora, Sp1 i Sp3, Rezultira njihov snažan učinak na DNMTs promotore uz izraženu njihovu transkripciju i stvaranje DNMTs proteina. Koja je funkcija DNMTs proteina? Kao komponente krucijalnog značaja u sustavima epigenetike ti proteini uvjetuju procese metilacije DNA molekula (adding the methyl group -CH3 to the molecules), generiraju nastanak 5-methyl cytosina (5mC) na template lancima DNA, te pad transkripcije metiliranih gena uz gašenje njihovih ekspresija. Zbog specifičnih uvjeta vezanih uz LRP1 i RAGE receptore, njihovi promotori imaju različite reakcije na DNMTs uvjetovanu metilaciju. Kod LRP1 metilacija promotora je brza i intenzivna, a kod RAGE promotora metilacija je izrazito usporena i smanjena. Time su i konačni učinci ta dva gena, odnosno njihovih receptora, izrazito različiti. Biokemijski i fiziološki procesi vezani uz LRP1 se postepeno usporavaju i prigušuju, a procesi vezani uz RAGE postaju sve više ekspresivniji. Druga grupa procesa vezana uz epigenetiku i programirano starenje obuhvaća oksidativnu demetilaciju 5mC segmenata DNA putem ten-eleven translocases (TET), thymine DNA glicosylase (TDG) i base excision repair enzymes (BER). Sve ukazuje kako je ova druga grupa zbivanja manje efikasna od prve grupe, te metilacija očito dominira. Da to priroda nije tako programirala, proces starenja bio bi nepredvidiv. Sve veći prodor misaonih analiza u bit procesa starenja, te eksperimentalni rezultati, sve više upućuju na presudnu važnost procesa transkripcije ranije prikazanih gena. Obnavljajuće transkripcije pod snažnom kontrolom Sp proteina, i njihovih u genomu lociranih programa, utiču na maksimalno moguće trajanje života individua neke vrste. Cilj ove studije je dati dodatna objašnjenja uloge spomenutih receptora u programiranom starenju živih bića. Dodatni cilj ove studije je prikaz najnovijih saznanja o specifičnoj blokadi, u staroj dobi, povećane ekspresije RAGE receptora, i ciljane genske terapije, također u staroj dobi, prigušene ekspresije LRP1 receptora.

Ključne riječi: Proces starenja, epigenetika, metilacija i demetilacija, LRP1 i RAGE receptori

Introduction

The aging process, essentially a natural, complex, inevitable, expedient and multi-profile process in the life cycle of living beings, therefore also of humans, according to today's knowledge, most likely takes place on the basis of a program located in the genome. Looking at the human being, this does not diminish the importance of dividing that process into normal or physiological aging and accelerated or pathological aging related to diseases. Programmed aging, according to the author of this study, is based on the assumption of the effects and control of two large transmembrane receptors, LRP1 (gene position 12q13.3) and RAGE (gene position 6p21.32), and three DNMTs (DNMT3A gene position 2p23.3, DNMT1 gene position 19p13.2, DNMT3B gene position 20q11.21) and two Sp proteins (Sp1, specificity protein 1, gene position 12q13.13, Sp3, specificity protein 3, gene position 2q31.1) becomes clearer after the involvement of epigenetics and reactive oxygen species (ROS – O_2^{*-} superoxide radical, H_2O_2 hydrogen peroxide, *OH hydroxyl radical) in this process[1-5].

In the presented study, a new hypothesis was put forward about the crucial role of the two large receptors, LRP1 and RAGE, in the process of programmed aging. Science has been dealing with the problem of programmed aging for many years. A detailed study of the theories known to this day about that process (a whole set of current theories try to explain the essence of this process. In addition to different variations of the programmed aging theory, on the other hand, actual are the theory of reactive oxygen species (ROS) [6], cross-linking theory of long protein molecules [7], mutation theory [8], autoimmune theory [9], free radical Harman's theory [10], microglial aging theory [11], mitochondrial theory of aging [12], non-enzymatic glycation theory that is the result of the effects of advanced glycation end products - AGEs compounds[13], and chronographic aging theory[14]) indicate that a solution has not yet been found. By analyzing the pathophysiology of Alzheimer's disease, which has been extensively written about, where there is an evident connection between the disease and the aging process, it was observed that in this disease, and even without it, the expression of LRP1 decreases with aging, while the expression of RAGE tends to increase [15,16]. This led the author of this study to think about the possible role of these two large transmembrane receptors in the process of natural programmed aging of living beings. Some examples of events in the LRP1 expression decrease during aging can be given: drop of proteases degradation, drop of lysosomal enzymes activity, drop of endocytosis, transcytosis and exocytosis, drop of the activity of cell signaling; drop of the phagocytosis of myelin debris and phagocytosis of apoptotic cells; the resulting drop of tissue cleaning from toxic and dangerous compounds and their accumulation in the cells. Drop in the prevention of cancer invasion. Rise in the possibility of cancer invasion. Something related to the RAGE receptor and its gene: age related decline of methylated cytosines in RAGE promoter, strong activation of nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase); rise of oxidative stress (accumulation of ROS - O_2^{*-}, H_2O_2, *OH); activation of NF-kB cascade (rise of PDGF, VCAM-1, ICAM-1, e-SELECTIN, MCP-1, M-CSF, COX-2, MMP-2 TNFα, IL-8, IL-6, IL-1), evident signs of atherogenesis, reinforced astrocyte dysfunction. RAGE dependent microglial activation, signs of chronic inflammation. These are just some of the moments related to the decrease in LRP1 expression and the increase in RAGE expression **(Table 1 and 2)**[17-19].

Further analysis of these events on the BBB related to AD and aging is presented by Doreen Osgood et al.[20]. At this site, Aβ-transport receptors, in fact specific proteins, LRP1 and RAGE, show significant alterations during aging. The expression of the efflux transporters LRP-1 and P-gp decreases, and the expression of the influx transporter RAGE increases. These receptors otherwise have important roles in maintaining biochemical homeostasis of the brain. They are also highly conserved throughout mammalian evolution. The authors of the paper admit that the current knowledge about how and why their expression in the BBB region is significantly altered during aging and in AD is very insufficient. The data suggest that gene transcription is altered with aging by some upstream events rather than a post-transcriptional, translational or direct effect on these cell surface receptors. Transcriptional alterations of gene expression may occur via modification of the gene promoter. One hundred male F344/BN hybrid rats with similar characteristics were used in this study. Analysis of graphs obtained from experiments on these mice, which show the correlation between the relationship between receptors and their mRNAs, indicates a close match between the descending curves for LRP1/age and the ascending curves for RAGE/age. Again, it is important to emphasize the modifications of gene promoters.

Table 1. Drop of the values (activity) of a range of important parameters linked with the LRP1 during ageing and age

Parameter	characteristics and functions
drop of proteases degradation; drop of lysosomal enzymes activity;	lysosomal enzymes (acid hydrolases) are responsible for breaking down complex chemicals within a cell. They contain about 40 types of hydrollytic enzymes including proteases, nucleases, glycosidases, lipases, sulfatases, phospholipases, and phosphatases;
drop of endocytosis; drop of transcytosis; drop of exocytosis; drop of the activity of cell signaling; drop of the phagocytosis of myelin debris; drop of the phagocytosis of apoptotic cells;	Drop of tissue cleaning from toxic and dangerous compounds and their accumulation in the cells;
drop in tumor invasion;	One of the most important LRP1 function is in clearing proteases such as plasmin, urokinase-type plasminogen activator, and metalloproteinases, which contributes to prevention of cancer invasion. LRP1 absance increases possibility of cancer invasion;
LRP1 deficiency in neurons;	Drop in insulin signaling, reduced levels of glucose transporters GLUT3 and GLUT4; drop in glucose uptake; rise in glucose intolerance; Disbalance in lipid homeostasis; Decline in cholesterol transport in the brain; Disbalance in regulation of cell proliferation, migration, apoptosis, and contraction of vascular cells, drop in maintaining the vascular homeostasis;
smooth muscle cells alteration;	Excess matrix deposition into the arterial wall,(smLRP1-/-) mice; medial thickening of the arterial vessels, aortic dilatation with disorganized and degraded elastic lamina; smLRP1 -/- mice contain a 4-fold increase in protein levels of high-temperature requirement factor A1 (HtrA1) which degrades matrix components and impairs elastogenesis with fragmentation of elastic fibers. LRP1 -/- mice in their vassel walls also have excessive accumulation of connective tissue growth factor (CTGF) which is a key mediator of fibrosis.
drop of LRP1 receptor level located on the microglial cell membranes;	microglia expresses the increassed pro-inflammatory signaling (pro-inflammatory cytokines); activation of both JNK and NF-kB signaling (NF-kB cascade);
High sensibility of LRP1 promoter to methylation;	Strong rise of LRP1 promoter methylation by ageing;

Endocytosis, biological process by which the extracellular materials are transported into intracellular compartmenta; transcytosis, transport of different biological material across the cell; exocytosis, biological process in which a cell transports different materials out of a cell; phagocytosis, process by which a special cells phagocytes ingest or engulf other cells or particles; myelin debris, material compossed of inflammatory and neurotoxic factors; apoptosis, the process of programmed cell death; urokinase-type plasminogen activator, uPA, serine protease; GLUT 3 and GLUT 4 , proteins responsible for transport of glucose across the plasma membranes; HtrA1, high-temperature requirement factor A1, serine protease, tumor suppressor.

Table 2. Moving of the values of o range of important parameters linked with RAGE during ageing and age

Parameter	characteristics and functions
Methylated cytosines in the RAGE promoter region;	⟶ It is found a significant age-related decline of methylated cytosine in the RAGE promoter region in the human parietal cortex (superior parietal lobule or supramarginal gyrus-APP promoter region). This reduction in the number of methylcytosines (5mC) at transcription factor binding sites increases the expression of RAGE , which may in turn play a role in the ageing of the brain.
Rise in Protein kinase C (PKC) activation;	⟶ Stronger activation of nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase); rise of oxidative stress (accumulation of ROS (O_2^{*-}, H_2O_2, *OH); activation of NF-κB cascade; elevation of PDGF, VCAM1, ICAM1, E-selectin, MCP-1, M-CSF, COX-2, MMP, TNF-α, Evident signs of atherogenesis. Reinforced astrocyte dysfunction, Consequence of the stronger oxidative stress;
AGEs binding with RAGE, AGE-induced RAGE overexpression;	⟶ RAGE dependent microglial activation, Signes of atherogenesis elevation, RAGE binding with damage-associated molecular pattern molecules (DAMPs): S100s, AGEs, HMGB1, and DNA; support of conditions of chronic inflammation, elevated RAGE signaling and induction of diabetic vascular complications, cardiovascular disease (CVD), cancer, Alzheimer's disease, and a range of inflammatory diseases, increased generation of oxygen radicals, activation of nuclear factor-kappa B, increased expression and release of pro-inflammatory cytokines; onset or accelerated course of atherosclerosis, coronary artery disease, hypertension, cerebral vascular disease, hyperthyroidism, Alzheimer's disease, end-stage renal disease, and diabetes mellitus.

AGE, advanced glycation end products; HMGB1, High mobility group box 1 protein, one of most important chromatin protein, mediator of inflammation and immune response; S100s, protein, included in signal transduction, cell differentation, transcription and cell cycle progression; DNA, deoxyribonucleic acid, molecular carrier of genetic informations; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells, transcription factor protein complex; VCAM-1, vascular cell adhesion protein 1, protein; PDGF, platelet derived growth factor,protein; ICAM-1, intercellular adhesion molecule1, protein; E-selectin, endothelial -leukocyte adhesion molecule 1; MCP-1, monocyte chemoatractant protein-1; M-CSF, macrophage colony-stimulating factor; COX-2, cyclooxygenase-2; TNF-α, tumor necrosis factor α;

Reactive oxygen species (ROS) and advanced glycation end products (AGEs)

The creation of reactive oxygen species (ROS) in the human body and the occurrence of oxidative stress is largely related to the formation and accumulation of so-called advanced glycation end products (AGEs), complex molecules resulting from the covalent bonding, of a sugar molecule, such as glucose or fructose, to a protein or lipid molecule (glycation or nonenzymatic glycosylation). This binding takes place slowly and without enzyme control. The creation of these endogenous compounds takes place slowly and permanently throughout life (Maillard reaction). Binding of AGE compounds to RAGE leads to activation of protein kinase C (PKC) and NADPH oxidase, with strong generation of ROS. Due to the effects of AGEs on mitochondria and the mitochondrial electronic transport chain, there are frequent damages to that chain with the escape of electrons (e^-), their joining with molecular oxygen (O_2), and the formation of ROS. On the other hand, with the intake of unhealthy food (food processing by dry heat, highly processed food, high lipid and high protein food-meat, cheese, eggs, high temperatures in food processing, grilling, broiling roasting) and exposure to various adverse external influences, for example: ultraviolet (UV) radiation, cigarette smoking, alcohol consumption, exogenous AGEs enter the body with subsequent harmful effects. In addition to harmful effects on the body, ROS, on the other hand, play an important role in intermolecular signaling and especially in the control of transcription factors [1,16].

Activation of Sp1 and Sp3 proteins

It has already been emphasized that Sp1 and Sp3 proteins are abundantly present in tissues and cells. Local ROS elements lead to strong activation, transcription and expression of these two factors (Sp1 and Sp3). The result is their strong effect on DNMTs promoters with pronounced gene transcription and formation of DNMTs proteins (DNMT1, DNMT3A, DNMT3B) (***Fig.1***)[20]. Liwei Wang, et al.[21], emphasize that Sp1 activity can be elevated by the fact that these stress factors (ROS) can activate the p42/p44 mitogen activated protein kinase pathway, and the c-Jun NH2 terminal kinase-related signaling pathway, both of which may be responsible for the Sp1 transcriptor overactivation. This leads to the consequent expression of multiple Sp1 downstream genes (for example DNMTs genes).

It is obvious that in the case of oxidative stress, the increased amount of ROS, along with the damage to receptors (LRP1 and RAGE) and to DNMTs proteins, also acts as a signaling pathway for the activation of Sp1 and Sp3 transcriptors. This is followed by the activation of LRP1, RAGE and DNMTs genes and their transcription. This is in fact a protective mechanism for creating new correct DNMTs proteins (***Fig.1***).

Guang Jia, et al.[22], reveal that c-Jun NH2-terminal kinase is strongly activated by a variety of stressful cellular environments, such as chemotherapy and oxidative stress. And this is where protein phosphorylation is important.

Kathryn Z Guyton, et al.[23], examining the influence of ROS on ERK1(p42) and ERK2 (p44), found that in both kinases, ROS leads to their strong activation, with direct phosphorylation (addition of phosphoryl-PO3-group to a molecule) of the Sp1 protein on its threonine 453 and 273. Thus activated Sp1 strongly activates many downstream genes (the human genome contains about 20,000 genes used to code proteins).

Julia Milanini, et al.[24], indicate that p42/p44 MAPK directly phosphorylates Sp1 on threonine 453 and 739 both in vitro and in vivo. Mutation of the mentioned positions halves the strength of transcriptional activity of Sp1.

Jin Wu, et al.[25], present the finding that oxidative stress, through activation of c-Jun NH2 terminal kinase, contributes to the proinflammatory phenotype of diabetic mesangial cells.

Linda Weis, et al.[26], indicate that c-Jun NH2-terminal kinases (JNKs) are a group of mitogen-activated protein kinases (MAP) that participate in signal transduction events important for specific cellular functions. Cellular stress and cytokines lead to the phosphorylation of JNK and its subsequent activation. Phosphorylation takes place on threonine and tyrosine residues (dual phosphorylation) within protein kinase subdomain VIII. The activation of JNK is conditioned by the activation of a wide range of stimulators, among them some growth factors, cytokines, UV-irradiation, heat shock, ceramide, and certain inhibitors of protein synthesis.

It is important to point out here that the human genome contains about 28 million CpG sites, of which about 60% are methylated at position 5 of cytosine (5mC) [27].

As can be seen from the previous presentation. two transcription factors, Sp1 and Sp3, are strongly activated by ROS with their enhanced expression and transcription. Increased concentration of Sp protein leads to strong transcription and expression of DNMTs genes, and generation, after translation in ER, of their transcripts, DNMTs protein. The basic function of DNMTs protein is the methylation of CpG sequences (adding a -CH3 or methyl group) in the promoters of a number of genes in the genome, among them LRP1 and RAGE genes. The methylation processes of RAGE and LRP1 differ significantly.

AGE/RAGE/cytokines molecular net

The LRP1 promoter is not protected by the AGE/RAGE/cytokines molecular network. Large molecules of DNMTs (the largest enzymes in humans, 1620 aa) easily approach the LRP1 promoter and perform intense methylation. In the case of the RAGE promoter, this approach is extremely difficult due to the comprehensive network of AGE/RAGE/cytokines, and methylation is weak. This is additionally contributed by the increasing

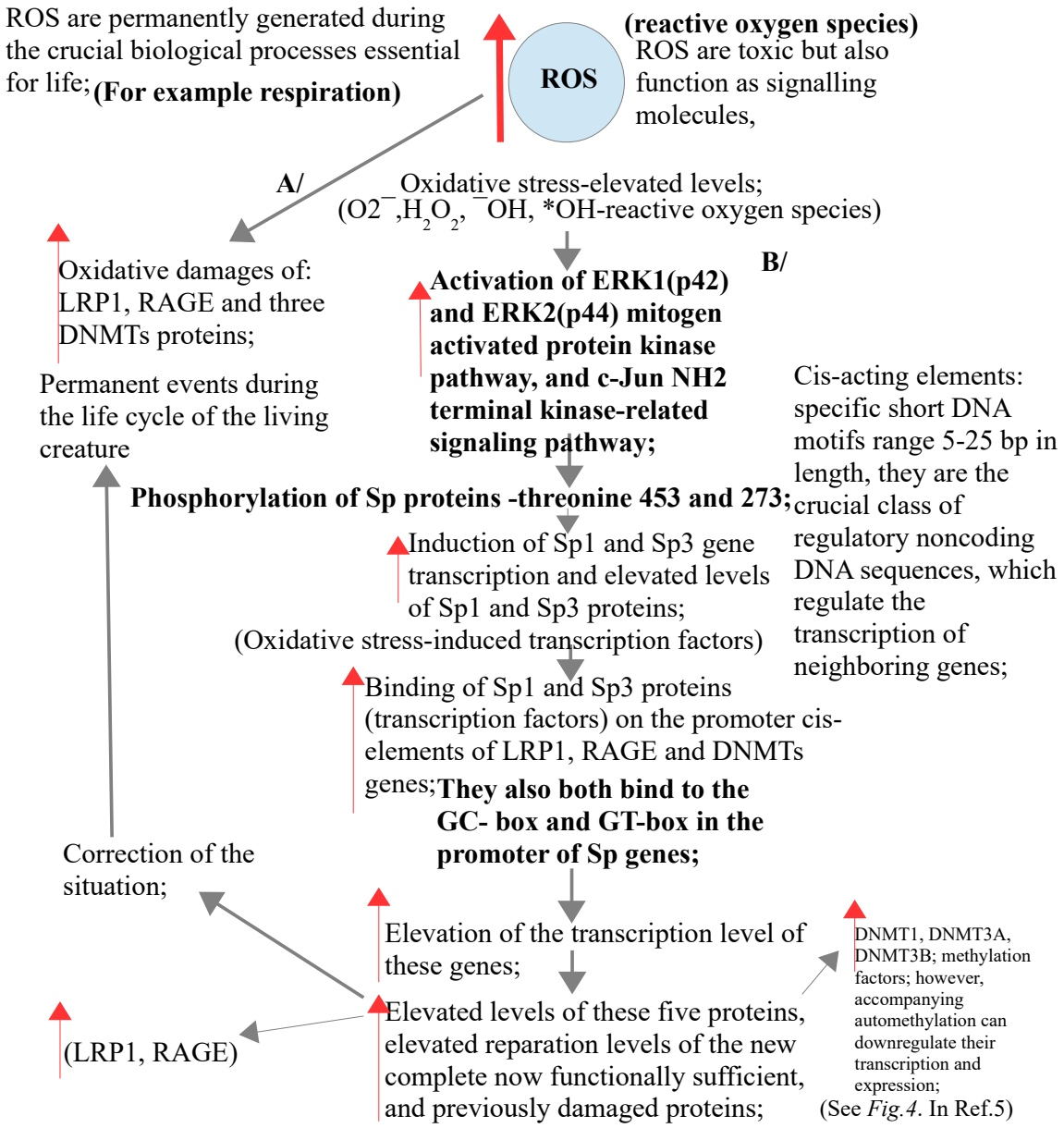

Fig.1. **Schematic presentation of the protective role of Sp1 and Sp3**

Sp1 and Sp3 transcription factors are zinc finger DNA-binding domain proteins that recognize the specific DNA-binding motifs GC-box (GGGCGGG) and GT-box (GGTGTGGGG), they are essential for the expression of DNMTs proteins; gene promoter cis-elements, specific short DNA motifs range from 5 to 25 bp in length, regions of non-coding DNA, regulators of the neighboring genes transcription; $O2^{-}$, superoxide, diatomic oxygen, an inorganic radical anion, a member of ROS; H_2O_2, hydrogen peroxide.

attraction of AGEs to RAGE (active form-there is not necessary the furin action in ER) during the life cycle, and their increasing accumulation around the RAGE promoter, especially in old age. Between AGEs and RAGE (active form) there is normally a strong mutual attraction and binding, which is not the case with LRP1. In addition, AGEs lead to a strong expression of RAGE through a positive feedback mechanism. As a result, the expression of LRP1 decreases with age, and the expression of RAGE increases [15] Vital processes related to the expression of LRP1 (LRP1 protein obtained by mRNA LRP1 translation become active by the furin action in ER) become more and more muted over time, and processes related to RAGE become more expressive. LRP1-induced processes are essential for vitality and longevity, and RAGE-related processes are pro-atherogenic in nature, blocking vitality and the possibility of longevity (**Table 1 and 2**)[6,13-18].

It is also necessary to pay attention to the following values of the mentioned proteins: DNMT1-size 1632 aa, molec. mass 185 kDa; DNMT3A-size 912 aa, molec. mass 102 kDa; DNMT3B-size 853 aa, molec. mass 95 kDa; LRP1-size 4544 aa, molec. mass 600 kDa; RAGE-size 320 aa, molec. mass 50-55 kDa; Sp1 protein-size 785 aa, molec. mass 81 kDa; Sp3-size 781 aa, molec. mass 82 kDa. Sp1 and Sp3 play an important role in cell growth, differentiation, apoptosis and carcinogenesis.

Sterile chronic inflammation and ageing

Experimental research and theoretical analyses clearly indicate that the process of programmed human aging is essentially related to signs of sterile chronic inflammation. What is inflammation? Inflammation is part of the biological response of body tissues to harmful stimuli such as pathogens, damaged cells and various irritants. Infection is the invasion and growth of microorganisms in the body. These can be bacteria, viruses or fungi. Inflammation can be due to infection (septic inflammation) or non-infectious agents. The process of programmed aging is essentially an aseptic non-infectious process. From the previous text it is evident that Sp1 and Sp3 are strongly involved in the pathophysiology of aseptic non-infectious inflammation.

Mengzhou Zhou, et al.[28], provides an exhaustive analysis of the so-called AGEs-RAGE axis and its implication in inflammatory pathologies. This axis has a decisive influence on the occurrence and development of chronic aseptic inflammation, crucial for the course of a whole series of chronic non-infectious diseases such as diabetes mellitus, atherasclerosis, cancer, but also programmed aging. AGEs and their receptor RAGE are key factors in these chronic events. AGE formation and accumulation are the result of the so-called Maillard reactions that occur by non-enzymatic interaction of reducing sugars (glucose, fructose) with the amino group of proteins, lipids or nucleic acids, the resulting Schiff base, Amadori rearrangement and oxidative modification generate the formation ofAGEs. AGE activates RAGE.

Activation of RAGE leads to biochemical events that, on a genetic basis, lead to Alzheimer's disease (AD) or determine the aging process. Activated PKC (electron donor), NADPH (NOX-2), and generated ROS participate here. All this results in the progression of the NF-kB cascade with the formation of PDGF, VCAM-1, ICAM-1, e-SELECTIN, MCP-1, M-CSF, COX-2, MMP-2, TNFα, IL-8, IL-6 and IL-1. The aforementioned platelet-derived growth factor (PDGF), adhesive molecules (VCAM-1, ICAM-1), enzymes and cytokines promote atherogenesis. The resulting ROS enhances the activation and transcription of both Sp genes (***Fig.2***).

The totality of physiological and biochemical processes essential for sustaining life, but also the simultaneous generation of ROS products is given (***Fig. 3***).

Fang Fang, et al.[29], in their research on the behavior of RAGE receptors on the membranes of neurons and microglia, found in these cells increased production of IL-1β and TNF-α, increased tissue infiltration of astrocytes and microglia, accumulation of Aβ, and reduced acetylcholine esterase (AchE) activity. Activated microglia leads to neuronal damage and neuroinflammation.

Aino Soro Paavonen, et al.[30], state that RAGE is a key mediator in the development of atherogenesis in the diabetic vasculature. They indicate the important role of RAGE activation in the development of diabetic nephropathy, neuropathy and impaired angiogenesis. RAGE also plays an important role in supporting proinflammatory and prothrombotic mechanisms. It also plays an important role in increasing the expression of NADPH oxidase, mitochondrial oxidative activity and suppressing endogenous antioxidant activity.

Ravichandran Ramasamy, et al.[31], indicate that the RAGE cytoplasmic tail binds to the formin Diaphanous 1 (DIAPH 1) and conditions AGE signaling. DIAPH1 is a protein member of the protein group called formin and has an important role in cytoskeletal rearrangement by nucleation of actin filaments. It is necessary for the formation of stress fibers, endocytosis in endosomes, and stabilization of microtubules and cellular migration.

Qing Yue, Yu Song, Zi Liu, et al. [32], claim that RAGE is a critical molecule in the onset and support of the inflammatory response. By binding to RAGE, its ligands trigger an intracellular signal cascade, act on intracellular signal transduction, stimulate cytokine secretion, and play a key role in the occurrence and development of immune-related diseases (eg Lupus erythematodes, rheumatoid arthritis, Alzheimer's disease). They are also active in inflammation, apoptosis, autophagy, and endoplasmic reticulum stress.

Laura M Senatus and Ann Marie Schmidt [33], already at the beginning of their presentation, say that the process of advanced glycation leads to the creation and accumulation of a heterogeneous group of molecules called advanced glycation end products, or AGEs. They are rapidly created in disorders such as diabetes,

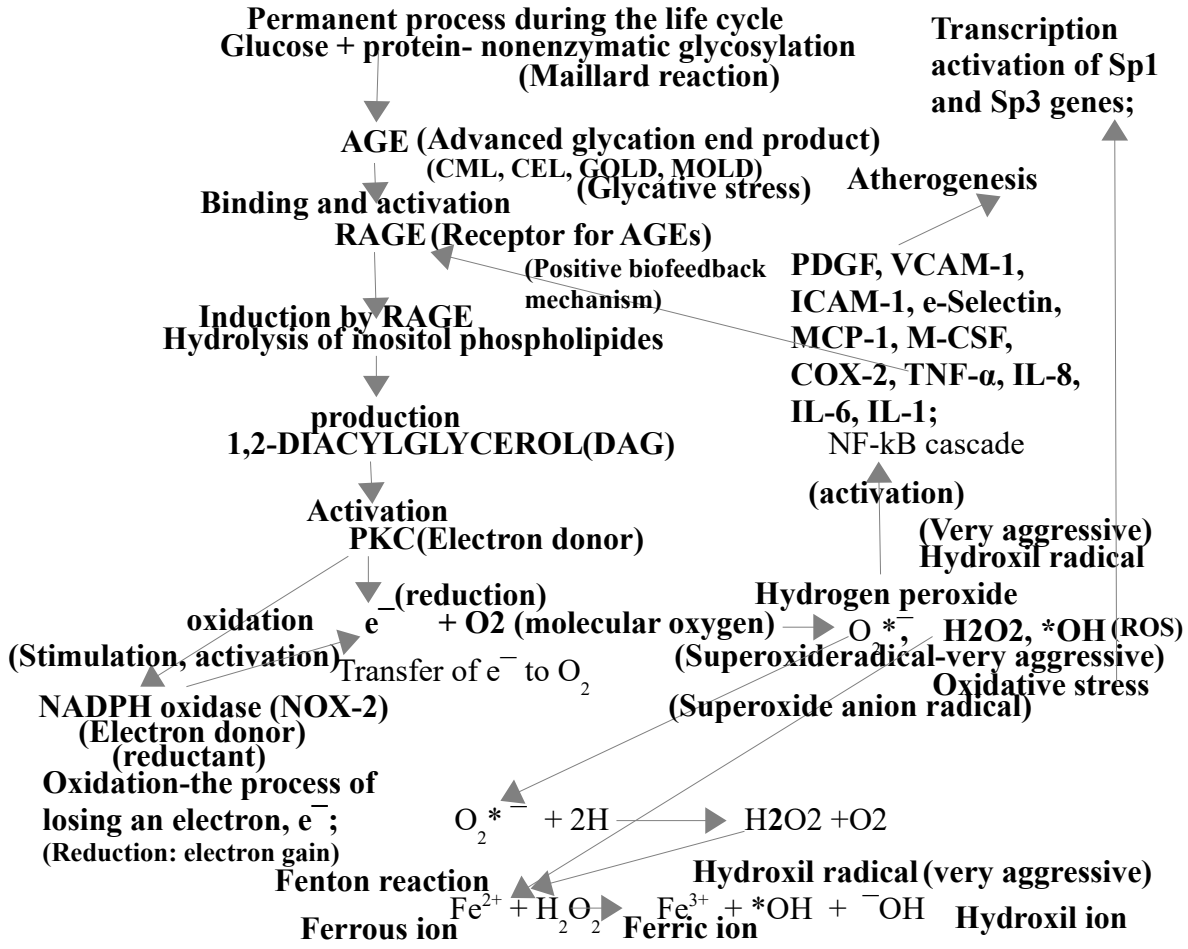

Fig. 2. Activation of NADPH oxidase and induction of oxidative stress

NADPH oxidase a family of enzymes whose function is to catalize the transfer of electrons to O_2 generating superoxide or H_2O_2 using NADPH as an electron donor. It is reductant. The 1,2-diacylglycerol (DAG) activates protein kinase C (PKC). Phosphatidylinositol (PI), also known as inositol molecule. One electron reduction of O_2 gives rise to superoxide anion radical (O2*-), which then undergones another one electron reduction to yield hydrogen peroxide (H_2O_2). One electron reduction of hydrogen peroxide generates hydroxyl radical (OH*), which can then be reduced by one electron to form water. AGEs, via RAGE stimulate endothelial cells to generate ROS and to activate cellular signaling pathways; glycative stress, biological stress induced by non-enzymatic glycation reaction, including AGE formation and accumulation, glycation-induced dysfunction of proteins, cellular signaling, inflammation, oxidation and tissue damage; *Nε*-(carboxymethyl)-lysine, CML; *Nε*-(carboxyethyl)-lysine, CEL; glyoxal-lysine dimer, GOLD; methyl-glyoxal-lysine dimer, MOLD.

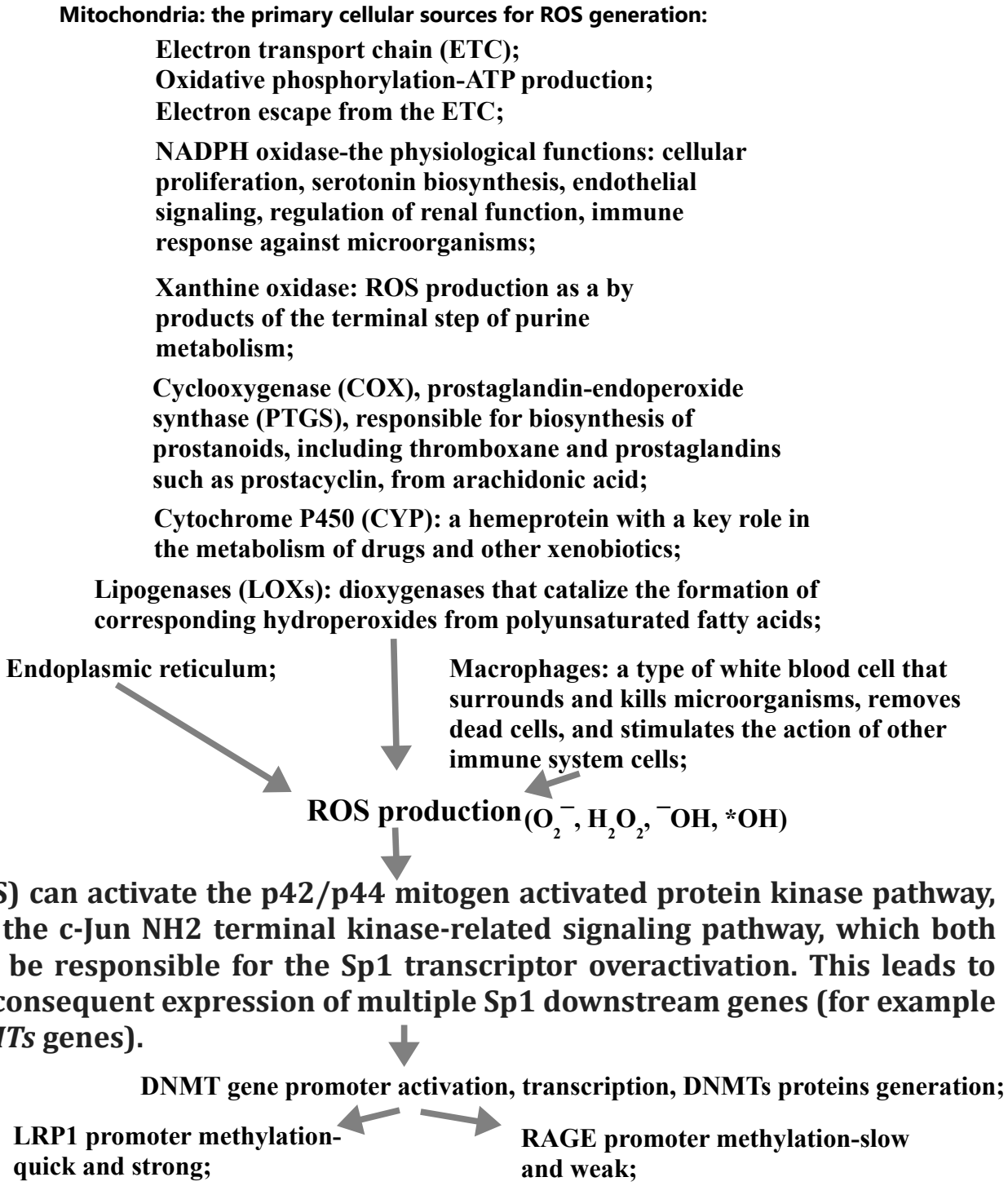

Fig. 3. **Endogenous ROS generation, Sp transcriptors overactivation, and LRP1 and RAGE methylation**

$O2^-$, superoxide , diatomic oxygen, an inorganic radical anion; H_2O_2, hydrogen peroxide; OH^-, hidroxil ion; OH*, hidroxil radical; ROS, reactive oxygen species; Sp1 and Sp3, transcription factors; DNMT, DNA methyltransferase.

renal disorders, inflammation, neurodegeneration, and aging (this refers to endogenous production). They are also found in certain foods and tobacco products (exogenous consumption). In the body, AGEs lead to cross-linking of long-lived molecules, such as collagen, thereby causing stiffening of vessels with hyperpermeability and loss of structural integrity. By binding to RAGE, AGE compounds stimulate a cascade of events that results in the loss of vascular and tissue homeostasis, which favors the development of cardiovascular disease.

Katrin Kierdorf, et al.[34], believe that RAGE is the key molecule that initiates and supports the inflammatory response. These authors think that RAGE forms the link between the innate and adaptive immune systems. RAGE is expressed on activated endothelium, where it mediates leukocyte adhesion and transmigration.

Another process closely related to oxidative stress and its harmful effects is the process of glycation or non-enzymatic glycosylation. In essence, it consists of covalent bonding of sugar molecules, such as glucose or fructose, with protein or lipid molecules. This crucially important biological process takes place in the organism of a living being slowly and continuously throughout the entire life cycle. No enzymes are needed for its development. In addition to the endogenous process that takes place in the structures of a living organism (endogenous glycation), the same process takes place outside the body (exogenous glycation). In the body, glycation leads to a relatively stable intermediate of the reaction. dysfunction of biomolecules and it does not need ATP for that. The glycation process begins with the joining of the amino group (NH_2) of the protein and the carbonyl group of the sugar molecule (C=O). The glycation process continues spontaneously and via Schiff base and enaminol conditions the formation of the Amadori product, an early glycation product. Oxidation of Amadori products produces 3-deoxyglucosone (3DG), glyoxal (GO), methylglyoxal (MGO), and further lipid peroxidation Nε-carboxymethyl-lysine (CML), Nε-carboxyethyl-lysine (CEL), glyoxal-lysine dimer (GOLD) and methyl-glyoxal-lysine dimer (MOLD). CML, CEL, GOLD and MOLD are typical AGE compounds. The Amadori product by oxidation gives 2,3-enediol, which turns into dicarbonyl, and this by oxidative cleavage (by ROS) gives CML and erythronic acid. CML binds to RAGE with its strong activation (***FIG.4***)[,34]. Katarina Zgutka, Marta Tkacz, et al. [35], already at the beginning of their work, they point out that aging is a complex process that includes numerous changes at the cellular, tissue, organic and whole-body levels. AGEs compounds are products of non-enzymatic reactions between reducing sugars and proteins, lipids or nucleic acids. They are created in physiological and pathological conditions. Their accumulation leads to severe cellular and tissue damage in various organ systems. An important harmful effect is their mediation in the creation of reactive oxygen species (ROS), cross-linking of extracellular matrix proteins, and pro-inflammatory cytokines. In addition to hyperglycemia, a number of exogenous factors, such as cigarette smoke, high levels of refined and simple carbohydrates in the diet, hypercaloric diets, foods cooked at high temperature, and sedentary lifestyle, induce AGE production. The authors point out two basic ways of harmful effects of AGEs compounds in the occurrence of various diseases and disorders. One way is through the binding of AGEs to the RAGE receptor, and the other through cross-linking ECM proteins and lipids. AGEs activate mitogen-activated protein kinase (MAPK), nuclear factor kappa B (NF-kB) and signal transducer and transcriptional activator (STAT) pathways. All this results in increased production of pro-inflammatory molecules, such as cytokines, chemokines, and acute-phase proteins (inhibitors or mediators of the inflammatory processes): C-reactive protein, α1-acid glicoprotein, haptoglobulin, fibrinogen, α1-antitrypsin, complement components C3 and C4. They play an important role in the occurrence of diabetes mellitus, cardiovascular diseases, atherosclerosis, neurodegenerative diseases (Alzheimer's disease), and some types of cancer. In human body AGEs accumulate during normal aging and age-related diseases.

Slowing down the aging process by FPS-ZM1 RAGE receptor blockade

Based on the necessity of developing efficient high-affinity Aβ/RAGE blockers that are safe and non-toxic, Deane R, et al.[36], performed a screening of a small molecular library and discovered new tertiary amides that blocked the Aβ/RAGE interaction with strong binding affinity. The subsequent synthesis of a second-generation library with the identification of a high-affinity RAGE-specific inhibitor FPS-ZM1, that binds to the V domain of RAGE, easily passes through the BBB, and inhibits the Aβ-induced cellular stress in RAGE expressing cells in vitro and in vivo. Bounding with RAGE (experiments with mice) in the brain, FPS-ZM1 inhibits β-secretase activity, Aβ production, microglia activity and the neuroinflammatory response (15-17 month-old APPsw/mice). There were no signs of toxicity or increased rate of infection.

Therapeutic dose was 1 mg/kg ip.

Huang J, et al.[37], have found that FPS-ZM1 is a promising therapeutic agent in human inflammatory diseases caused by oral bacteria.

Yan Hong, et al.[38], in experiments with APPsw/0 transgenic mice, applied the intrahippocampal injections of AGEs, and after this administration they found signs of inflammation, oxidative stress, and increased escape latency of rats in the Morris water test. All of these are significantly reduced by FPS-ZM1 treatment.

Lan Wang, et al.[39], after intraperitoneal (i.p.) application of LPS 5mg/kg they have found in the hippocampus of C57BL/ mice overproduction of microglial pro-inflammatory cytokines IL-1β and TNF-α. It was found that FPS-ZM1 downregulated LPS-mediated increases in the phosphorylation levels of JAK/STAT-both in vivo and in vitro.

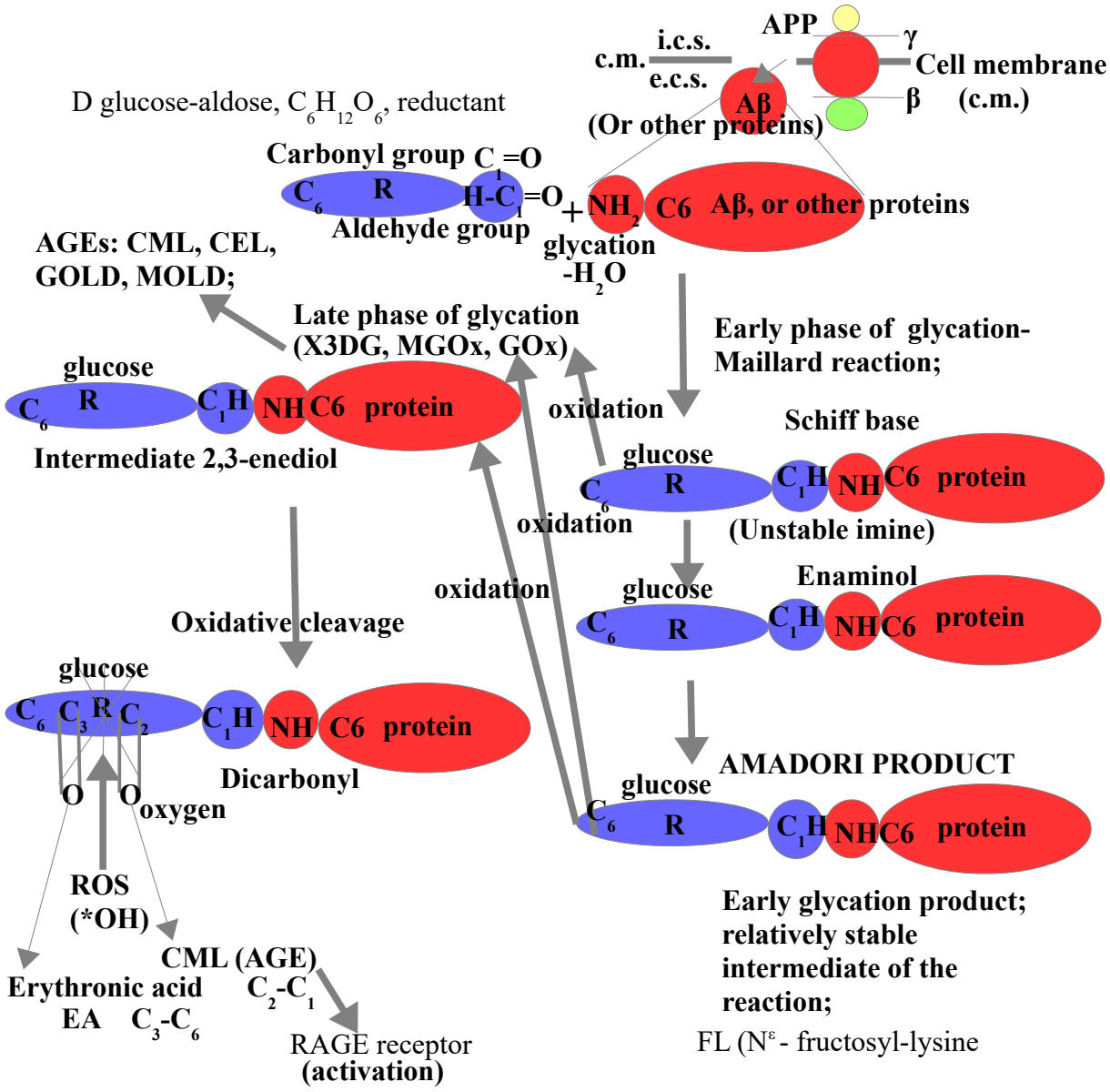

Fig. 4. **Schematic diagram of the glycation (nonenzymatic glycosylation) of proteins**

Glycation, sometimes called non-enzymatic glycosylation is the result of the covalent bonding of a sugar molecule, such as glucose or fructose ,to aprotein or lipid molecule, without the controlling action of an enzyme. It may occur either inside the body (endogenous glycation) or outside the body (exogenous glycation). It is haphazard process that impairs the functionining ofbiomolecules and does not require the expenditure of ATP. Protein glycation starts with a none nzymatic reactiobetween a sugar aldehyde or ketone, with a free amino group of amino acid. It leads, connected with the activated RAGE receptor to PKC activation, NADPH oxidase activation, ROS production, and NF-kB cascade, X3DG, 3-deoxyglucosone; GOx, glyoxal; MGOx, methylglyoxal; AGEs, advanced glycation end products; CML, Nε-(carboxymethyl)-lysine;CEL Nε -(carboxyethylethyl)-lysine; GOLD, glyoxal-lysine dimer; MOLD, methyl-glyoxal-lysine-dimer;

Yanian Kong, et al.[40], already at the beginning of their study, pointed out that strong expression of RAGE receptors is extremely harmful to the human body. By causing a cascade of biochemical events, this expression leads to the appearance of Alzheimer's disease (AD) and its accelerated development. The trigger for these adverse events is the fusion of RAGE and AGEs molecules. The aforementioned cascade leads to strong activation of PKC and NADPH. This is followed by strong oxidative stress, NF-kB cascade and dysfunction of astrocytes. On the other hand, hyperphosphorylation of tau protein and the development of neurofibrillary tangles (NFT) occur. RAGE inhibitors, among them FPS-ZM1 in particular, strongly block the binding of ligands to RAGE and thereby prevent the aforementioned cascade and its consequences. The authors of this study believe that FPS-ZM1 can be used as a disease modifying agent for AD.

Slowing down the aging process by *LRP1* gene therapy

Sagare, et al.[41], point out that the LRP1 expression at the BBB is reduced during normal aging. This reduction can be corrected by lifestyle changes, pharmacological agents, and gene therapy that has a crucial role. Effective gene transfer to BBB can be achieved by non-viral and viral systems. Viral based systems are more effective in mediating cell entry and transfer of genes to endothelial BBB cells. Adeno-associated virus (AAV) is optimal for achieving long-term gene expression in these cells. It is unable to replicate autonomously, which excludes pathogenicity. In direct targeting to the BBB endothelial cell genes, the important function has a small peptide (7-9 aa long) that has been inserted into the viral capsid sequence (protective coat surrounding the viral genome) to modify viral tropism. So, it is necessary to identify molecular signature epitopes in the cerebral endothelial cells of the aging brain, and present these epitopes by the mentioned analogous small peptides on the capsid of AAV to enhance site-specific distribution after intravenous injections. In this way, it would be possible to use AAV-2 carrying the cDNA of LRP1 (complementary DNA-contains only coding sequences), or LRP1 smaller fragments to restore reduced LRP1 expression in vascular endothelial cells in the aging brain.

Angeliki Maria Nikolakopoulou, et al.[17], in their study present a number of interesting facts related to the endothelial LRP1 protective role against neurodegeneration by inhibiting the proinflammatory cyclophilin A-matrix metalloprotenase-9 pathway at the BBB. They emphasize that endothelial LRP1 gene replacement therapy, in the presence of endothelial LRP1 loss, can prevent or reverse the development of neurodegeneration. Experiments with mice show that LRP1 endothelial knockout, with CypA (abundant intracellular pro-inflammatory cytokine) activation, can be restored with brain endothelial-specific in vivo LRP1 gene therapy. It is important to note that cyclophilins have a crucial function in protein folding, signaling, nucleic acid interaction, protein degradation, apoptosis, and in response to different stress stimuli, overexpression of CypA is also linked with aging. It is a potential atherogenic cytokine as well as a potential promoter of cardiac hypertrophy.

Ramanathan A, et al.[42], in a detailed study on the problem of reduced expression of the LRP1 receptor in AD and old age, express their opinion on the selective targeting of this receptor through the delivery of gene transfer vectors. Viral mediated gene transfer methods, particularly by the adeno-associated viral (AAV) system, have been proven effective in a number of peripheral cellular types, as well as in the CNS. The LRP1 receptor is particularly suitable for intravenously used targeted gene therapy due to its favorable location on the abluminal endothelial membrane of BBB cells. The animal models used were successfully tested by the AAV-2 vector system, using peptides with a strong affinity for cerebral vasculature. Recently, serotype AAV-9 has been shown to be particularly effective in the endothelial transduction at the BBB. These latest techniques used LRP1 whole cDNAs (complementary DNA, in genetics cDNA is DNA that is reversely transcribed from an RNA), or parts of their domains. Very good results have been obtained. The use of *LRP1* gene therapy on liver hepatocytes has also been shown to be effective**.**

Shaza S Issa, et al.[43], emphasize that advances in genetic engineering have enabled the development of effective gene therapy methods for a number of diseases based on adeno-associated viruses (AAVs). In their study they present a review of AAV discovery, properties, different serotypes, tropism, and uses in gene therapy of different diseases. However, they point out that today, there is a small number of approved AAV-based gene therapy medications**.**

Michael F Naso, et al. [44], declare that the Adeno-associated virus (AAV) is a non-enveloped virus that can be engineered to deliver DNA to target cells. Today, it is possible to generate in the laboratory the recombinant AAV viral particles, without original AAV genome, but with the incorporated DNA (cDNA) sequences of interest for various therapeutic applications. Consequently, the recombinant DNA (cDNA) is a form of DNA which is generated by a artificial way.

Berislav V Zlokovic, et al.[45], already point out at the beginning that low-density lipoprotein receptor-related protein-1 (LRP1), a member of the large low-density lipoprotein receptor family, is strongly involved in cellular transport of cholesterol, endocytosis of numerous ligands, transcytosis of ligands across the blood brain barrier (BBB), and transmembrane and nuclear signaling. LRP1 has been found to regulate brain and systemic clearance of Alzheimer's disease (AD) amyloid β-peptide. The authors point out that gene therapy strategies aimed at increasing LRP1 levels in the cerebrovascular system and in the BBB may have the potential to alleviate initial vascular damage by reducing Aβ accumulation during hit 1 and hit 2 states of AD pathogenesis.

Steffen E Storck, et al.[46], point out that brain endothelial LRP1

ablation results in protease-mediated tight junction degradation, P-glycoprotein (P-gp) reduction and a loss of BBB integrity. Deletion of LRP1 increases levels of cyclophilin A (CyPA, a ubiquitously distributed immunophilin protein), which increases metalloproteinase-9-mediated tight junction protein degradation and paracellular brain penetration of blood proteins, as well as neuronal damage. LRP1 gene therapy targeting the BBB partially corrects vascular leakage, neuronal damage, and behavioral deficits in mice.

Some interesting facts about the physiology and biochemistry of AAV-2 virus are presented in the paper by Jalish M Riyad and Thomas Weber,[47].

Conclusion

Aging is a natural, complex, multifaceted, inevitable and irreversible process. Its cause is still unknown. Aging essentially consists of two mutually closely related components: physiological or normal aging and accelerated or pathological aging related to diseases. Physiological aging, according to a series of indications, occurs due to a certain program in the genome. Several prominent proteins play a crucial role in this: LRP1, RAGE, three DNMTs and two Sp. Due to the different methylation conditions of their promoters, LRP1 is methylated faster and more strongly, while RAGE is methylated more slowly and weakly. This causes a faster weakening of LRP1 expression and the entire complex of physiological and biochemical events related to this receptor during life, and especially in the periods of aging and old age. Conversely, RAGE expression increases during aging, and thus a series of pro-atherogenic events accelerates, all of which results in the acceleration of programmed aging.

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Chest Radiography in the Intensive Care Unit - Need to Know Emergency Findings

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Abstract

Conventional chest radiography, also known as a chest X-ray, is a fundamental imaging tool for diagnosing cardiopulmonary disorders in critically ill patients within the intensive care unit (ICU). The interpretation of these radiographs is complicated by intricate radiological features and the frequent presence of lines, tubes, and other monitoring devices, which can obscure critical findings. Many cardiopulmonary conditions exhibit similar radiographic signs, such as airspace opacification, which complicates diagnosis. Typically, emergency findings on ICU chest X-rays include misplaced lines and tubes, iatrogenic pneumothorax and pneumomediastinum, congestive heart failure, pleural and pericardial effusions, foreign bodies, atelectasis, and various forms of pneumonia. Recognizing these conditions promptly is essential for effective patient management. This review article aims to explore the common emergency findings observed in ICU chest radiographs, shedding light on the complexities that healthcare professionals face in diagnosing and treating cardiopulmonary disorders in critically ill patients.

Keywords: Radiographic interpretation, Intensive Care Unit (ICU), Chest radiography (X-ray), Cardiopulmonary disorders, Emergency findings

Sažetak:

Radiogrami torakalnih organa u jedinicama intenzivnog liječenja – hitni nalazi koje je važno prepoznati

Konvencionalni radiogrami torakalnih organa, poznati i kao RTG toraksa, temeljni su dijagnostički alat za dijagnosticiranje kardiopulmonalnih poremećaja u kritično bolesnih pacijenata u jedinicama intenzivnog liječenja. Interpretacija snimki otežana je kompleksnim radiološkim značajkama te čestim prisustvom brojnih katetera, drenova te naprava za monitoriranje pacijenta koji mogu prikriti važne nalaze. Mnoga kardiopulmonalna stanja pokazuju slične radiološke znakova poput opacifikacije plućnog parenhima, što otežava postavljanje dijagnoze. Tipični hitni nalazi na radiogramima torakalnih organa iz jedinica intenzivnog liječenje uključuju pogrešno postavljenje katetere i tubuse, jatrogeni pneumotoraks i pneumomedijastinum, kongestivno srčano zatajenje, pleuralni i perikardni izljev, strana tijela, atelektazu, i razne vrste pneumonije. Promptno prepoznavanje ovih entiteta od iznimne je važnosti za učinkovito liječenje pacijenata. Ovaj pregledni članak donosi neke od najčešćih hitnih nalaza na radiogramima torakalnih organa iz jedinica intenzivnog liječenja te pruža uvid u izazove s kojima se radiolozi i drugi liječnici susreću pri dijagnosticiranju i liječenju kardiopulmonalnih poremećaja u kritično bolesnih pacijenata.

Ključne riječi: radiološka interpretacija, jedinice intenzivnog liječenja, radiogrami torakalnih organa, kardiopulmonalni poremećaji, hitni nalazi

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This article was submitted to RAD CASA - Medical Sciences as the review article

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 2 May 2025
Accepted: 29 May 2025
Published: 25 June 2025

Citation:
Mandić Paulić T, Coce N, Filipović-Grčić L, Paulić I, Tičinović N, Prutki M. Chest Radiography in the Intensive Care Unit - Need to Know Emergency Findings

567=70-71 (2025): 92-100
DOI: 10.21857/y26kecg4l9

Introduction

Chest radiography (X-ray) is a key tool for diagnosing cardiopulmonary disorders in ICU patients, but interpreting these X-rays presents several challenges. High imaging volume, variable image quality from portable X-rays, and the complexity of overlapping radiological features of different pathologies make diagnosis difficult. Using supine anteroposterior (AP) views instead of the ideal posteroanterior (PA) views, along with superimposed medical devices like catheters, tubes, and monitors, further complicates interpretation. Misplaced devices can also obscure critical findings or even cause complications. To improve diagnosis, optimal radiographic techniques, timely image access, and advanced technologies, such as deep learning algorithms employed by atrificial intelligence (AI), can support accurate and rapid interpretation, leading to better patient outcomes [1-4].

Review article

This review article will address several of the most prevalent emergency pathological conditions observed in ICU chest radiographs. These include cardiogenic and noncardiogenic pulmonary edema, pulmonary aspiration disease, atelectasis, pleural effusion, pneumothorax, pericardial effusion, pneumopericardium, misplaced lines, tubes, and other medical devices, as well as diaphragmatic hernia.

Cardiogenic and noncardiogenic pulmonary edema

Cardiogenic pulmonary edema, a frequently observed entity in ICU chest radiographs, exhibits a spectrum of imaging characteristics facilitating its prompt identification and accurate diagnosis. It originates from the gradual extravascular movement of fluid into the pulmonary interstitium and alveoli. Distinct imaging features mark its progression. Early manifestations include pulmonary venous hypertension, which is evident through the enlargement of the vascular pedicle width and redistribution of blood into upper lobe vessels. With hydrostatic pressure elevation to 20-25 mmHg, fluid translocates from intravascular spaces to the surrounding interstitium, leading to thickening of the interlobular fissures, peribronchovascular cuffing, and blurring of the pulmonary vessel walls (Figure 1). Subsequently, alveolar flooding occurs, manifesting as perihilar opacities (''batwing sing", Figure 2). Furthermore, less commonly seen noncardiogenic pulmonary edema shares similar imaging characteristics. This condition can arise from direct lung damage, such as pneumonia or inhalational injury, resulting in acute respiratory distress syndrome (ARDS) (Figure 3). Also, indirect causes may include conditions like acute pancreatitis, sepsis, severe trauma with shock, lung injury caused by mechanical ventilation, and transfusion-related acute lung injury (TRALI) [5].

Figure 1: Heart failure - cardiogenic interstitial pulmonary edema. In the context of heart failure-induced cardiogenic interstitial pulmonary edema, Figure 1 displays bilaterally prominent interstitial lung markings and peribronchovascular cuffing. Additionally, minimal pleural effusion was observed. Cardiomegaly is evident, along with an increased width of the vascular pedicle.

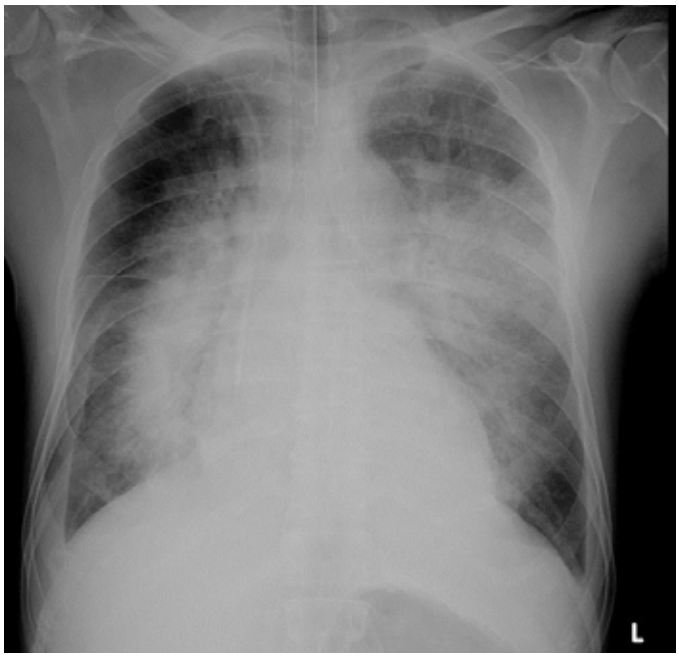

Figure 2: Heart failure - cardiogenic alveolar pulmonary edema. In the context of heart failure-induced cardiogenic alveolar pulmonary edema, Figure 2 displays bilateral symmetrical perihilar patchy lung shadowing, commonly referred to as the "batwing sign." The presence of cardiomegaly and bilateral pleural effusions is notable. Moreover, both the endotracheal tube and central venous line are appropriately positioned.

Figure 3: Acute Respiratory Distress Syndrome (ARDS) – Noncardiogenic Alveolar Pulmonary Edema. The babygram of a newborn reveals bilateral diffuse lung opacities with visible air bronchograms, indicative of acute respiratory distress syndrome (ARDS). No pleural effusion or heart enlargement is observed. The positioning of the endotracheal tube, peripherally inserted central catheter (PICC), and nasogastric tube is correct.

Figure 4: Inhaled foreign body. A triangular-shaped, mineral-density foreign body resembling a "tooth" is observed in the projection of the right main bronchus. No signs of overinflation or atelectasis were noted. Additionally, the nasogastric tube appears to be correctly inserted.

Pulmonary aspiration disease

Aspiring solid or liquid substances into the lower airways can lead to various pulmonary complications, including lobar or segmental pneumonia, bronchopneumonia, lung abscess, and empyema. These conditions typically affect the posterior segment of the upper lobes and the superior segment of the lower lobes. Among adults, alcoholism stands out as a significant predisposing factor, along with general anesthesia, loss of consciousness, and structural abnormalities of the pharynx and esophagus. The clinical and radiologic presentations of these pulmonary complications vary widely, ranging from asymptomatic focal inflammatory reactions with minimal radiologic abnormalities to severe, life-threatening diseases. In children, foreign body aspiration is a common occurrence, often resulting in obstructive lobar or segmental overinflation or atelectasis. Notably, the foreign body may not be visible on chest radiographs due to a lack of radiopacity (Figure 4).

In cases of massive gastric content aspiration, patients may exhibit an extensive, patchy broncho-pneumonic pattern on radiographs (Figure 5). Aspiration of infectious material can result in necrotizing consolidation and abscess formation within the lungs (Figure 6) [6].

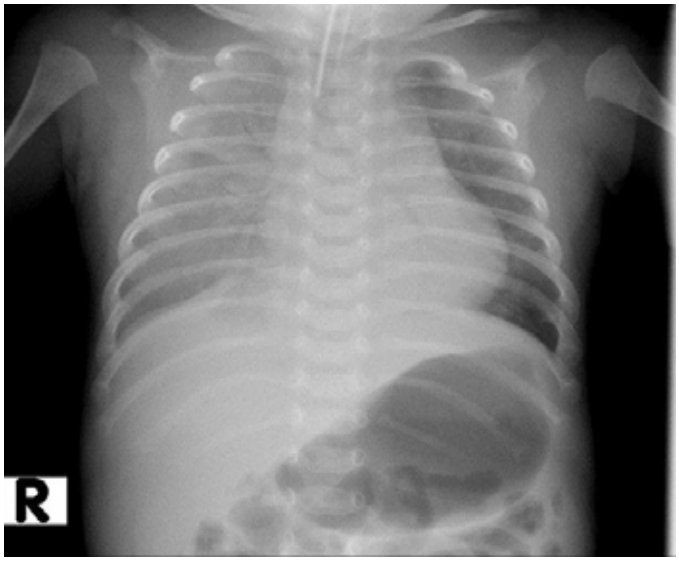

Figure 5: Aspiration pneumonia. The right lung parenchyma exhibits complete homogeneous shading, indicative of right-sided aspiration pneumonia, with visible air-bronchograms. The endotracheal tube is appropriately positioned. No evidence of pleural effusion or cardiomegaly was observed.

Figure 6: Pulmonary abscess. A cavitary lesion is identified in the right lung, characterized by a thick wall and an air-fluid level, surrounded by irregular parenchymal opacities. No indications of pleural effusion or cardiomegaly were observed.

Atelectasis

Lung collapse, also known as atelectasis, refers to the diminished inflation of all or part of the lung. It occurs due to various mechanisms. One of the most common is the loss of aeration and subsequent resorption of air distal to an obstructed airway, leading to decreased lung volume. When atelectasis affects only part of a lung lobe, it is termed partial or linear atelectasis, while the involvement of an entire lobe is referred to as lobar atelectasis [7]. Several factors can contribute to atelectasis, including endoluminal masses such as bronchogenic carcinoma, external compression from enlarged lymph nodes, mucus impaction seen in conditions like asthma or cystic fibrosis, foreign body inhalation (especially in young children, Figure 4), and inadequate ventilation, often due to a misplaced endotracheal tube (Figure 7).

Figure 7: Atelectasis. A misplaced endotracheal tube, with its tip erroneously positioned in the main right bronchus, leads to atelectasis of the upper right lung lobe. Concurrently, left perihilar incipient homogeneous opacities with air bronchograms indicate the onset of left lung atelectasis formation. The peripherally inserted central catheter (PICC) and nasogastric tube are positioned correctly.

A collapsed lung appears denser than normally inflated lung tissue, and the so-called "silhouette sign" (loss of the structure's outline) is a crucial imaging finding that suggests atelectasis, necessitating further evaluation [8].

Pleural effusion

Pleural effusion, a common occurrence among ICU patients, arises from disrupted fluid dynamics in the pleural space and often complicates various life-threatening conditions such as heart failure, fluid overload, hypoproteinemia, infection, pulmonary embolism, surgery, neoplastic disease, subphrenic inflammatory processes, trauma, and ascites. These effusions can vary in chemical composition, ranging from transudates to exudates, blood, chyme, and pus. However, discerning between these compositions is impossible on a supine chest radiograph, the primary imaging modality for ICU patients. Radiographic features of pleural effusion on a supine chest X-ray are nonspecific and pose challenges in analysis. Gravitational-conducted accumulation of pleural fluid, particularly in volumes exceeding 300 ml, typically manifests as posterobasal opacities.

Typical radiographic findings include loss of diaphragm contour with homogenous opacification towards the lung base, blunting of costophrenic angles, elevated hemidiaphragm with lateral dome displacement, homogenous density increase at the lung apex (known as "pleural cap") and along interlobar fissures with a characteristic biconvex shape, and widened mediastinum (Figure 8).

Figure 8: Pleural effusion. Bilaterally homogeneous opacification extending towards the lung base is observed, accompanied by effacement of the costophrenic angles. Additionally, prominent interstitial lung markings and peribronchovascular cuffing are noted. Furthermore, cardiomegaly is evident with an increased vascular pedicle width.

In cases of inconclusive findings, additional imaging, such as lateral decubitus films, may be performed to differentiate between loculated and free effusions. Lateral decubitus films are acquired when the patient is positioned on the side suspected of having an effusion, facilitating visualization of position-dependent fluid accumulation [9].

Iatrogenic pneumothorax

Pneumothorax is characterized by the presence of free gas in the pleural space. It can manifest in several forms: primary spontaneous, secondary spontaneous (occurring in the presence of underlying lung disease), and iatrogenic (resulting from invasive procedures such as pulmonary needle biopsy, central venous line placement, positive pressure ventilation, and other thoracic and abdominal procedures). Among these, iatrogenic pneumothorax is a potential consideration in ICU patients experiencing sudden pleuritic pain, dyspnea, tachypnea, and tachycardia. As a complication, tension pneumothorax may arise when pleural space pressure remains positive throughout the respiratory cycle [10]. In supine ICU patients, a typical radiological feature of pneumothorax is the visualization of a visceral pleural line devoid of distal lung markings, particularly prominent at the lung bases, leading to enlargement of the costophrenic angle, known as the "deep sulcus sign" (Figure 9).

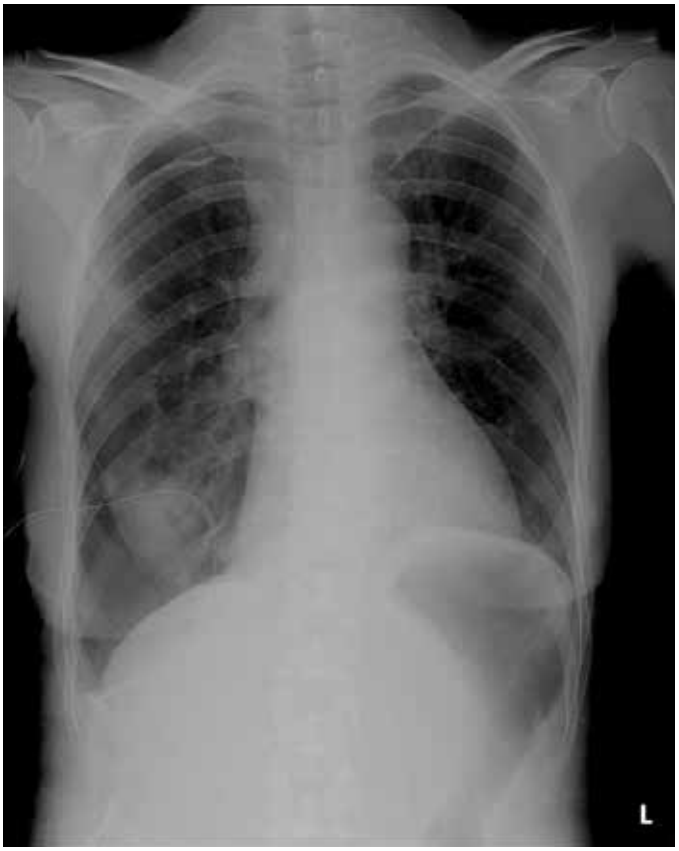

Figure 9: Pneumothorax. The radiograph reveals increased transparency of the enlarged right costophrenic angle, known as the "deep sulcus sign", which is indicative of a pneumothorax. It is accompanied by visualizing a visceral pleural line without distal lung markings. Additionally, there is a blunted appearance at the tip of the right costophrenic angle, suggesting the presence of minimal ipsilateral pleural effusion or hydropneumothorax. Notably, a chest tube is correctly positioned at the right costophrenic angle.

A pneumothorax is deemed significant when the distance from the parietal pleural surface to the lung edge exceeds 2 cm, warranting drainage intervention. However, it is crucial to differentiate genuine pneumothorax from artifacts such as skin folds, companion shadows, scapulae, or previous lung surgery, which may mimic its appearance on radiographs. Immediate post-treatment radiographs are vital for detecting complications and ensuring satisfactory positioning of tubes or lines [11, 12].

Pericardial effusion and pneumopericardium

The average volume of physiological pericardial fluid is typically less than
50 ml. Effusions smaller than 200 ml are often not visible on chest radiographs. The most sensitive indicator of pericardial effusion on a chest radiograph is an enlargement of the cardiac silhouette, characterized by a cardiothoracic ratio exceeding 50%. While this criterion demonstrates reasonable sensitivity (71%), its specificity is relatively low (41%). Pericardial effusion typically symmetrically expands the pericardial contour, resulting in a "globular" configuration, commonly referred to as the "water bottle sign" (Figure 10) [13].

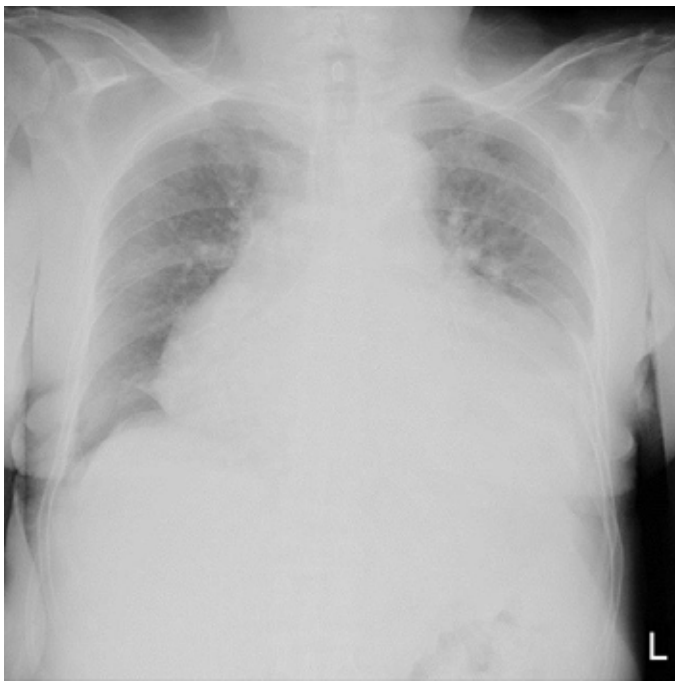

Figure 10: Pericardial effusion. A markedly enlarged heart with a globular contour typically indicates pericardial effusion, often referred to as the "water bottle sign." Concurrently, bilateral peribronchovascular cuffing accompanied by prominent interstitial lung markings suggests the presence of heart failure.

Figure 11: Pneumopericardium. The presence of a radiolucent zone surrounding the heart, commonly known as the "halo sign," along with a continuously delineated left diaphragm, indicates pneumopericardium. Additionally, inhomogeneous perihilar opacities are noted in the right lung. The central venous line is appropriately positioned. Sternotomy wires and epicardial electrodes are also visible.

Pneumopericardium signifies the loss of pericardial integrity, resulting in the presence of free air within the pericardial sac. In ICU patients, typical causes of pneumopericardium include invasive procedures, such as thoracic surgery and positive pressure ventilation. Small amounts of free air in the pericardium may not be visualized on chest radiographs. However, when present in sufficient volume, the distinct radiolucent zone surrounding the heart becomes evident, known as the "halo sign." Radiographically, the primary challenge is distinguishing pneumopericardium from the more prevalent pneumomediastinum. One useful differentiating feature is the so-called "continuous left diaphragm sign" (Figure 11). Additionally, similar to pericardial effusions, a large pneumopericardium has the potential to cause tamponade, resulting in the "small heart sign," characterized by a sudden reduction in cardiac diameter exceeding 2 cm [14].

Misplaced lines, tubes, and other devices

ICU patients require continuous monitoring of vital functions and mechanical and pharmacological support provided through various devices, including central venous lines, Swan-Ganz lines, pacemaker electrical leads, left ventricular assistance devices, endotracheal tubes, chest tubes, and nasogastric tubes. Chest radiographs play a central role in ensuring the correct positioning of these devices and monitoring for common post-placement complications. The ideal positioning of correctly implemented lines, catheters, devices, and tubes on chest radiographs is as follows:

- Central venous line: The tip should be positioned in the superior vena cava, slightly above the right atrium (Figure 12).
- Swan-Ganz catheter: The tip should be positioned in the right or left main pulmonary artery.
- Pacemaker electrical leads: The tips of the leads should project against the right atrium and ventricle. In the case of implantable cardioverter-defibrillators (ICDs) or biventricular pacemakers, the tips of the leads should project against the right atrium and both ventricles (Figure 13).
- Left ventricular assistance device: Inserted into the ascending aorta, passing through the aortic valve, with the tip positioned in the left ventricle.
- Endotracheal tube: The tip should be positioned approximately 5 cm ± 2 cm from the carina at the T3 or T4 vertebral body level.
- Chest tube: In cases of pneumothorax, the tip should be positioned apically to facilitate the evacuation of gas. For pleural effusion, the tip should be placed basally for fluid drainage. In instances of loculated pleural fluid, the tip should be positioned in the specific location of the loculation.
- Nasogastric tube: The tip should be positioned within the stomach, extending beyond the cardia.

These guidelines help ensure proper device function and reduce the risk of complications in ICU patients, facilitating effective management and care [15-18].

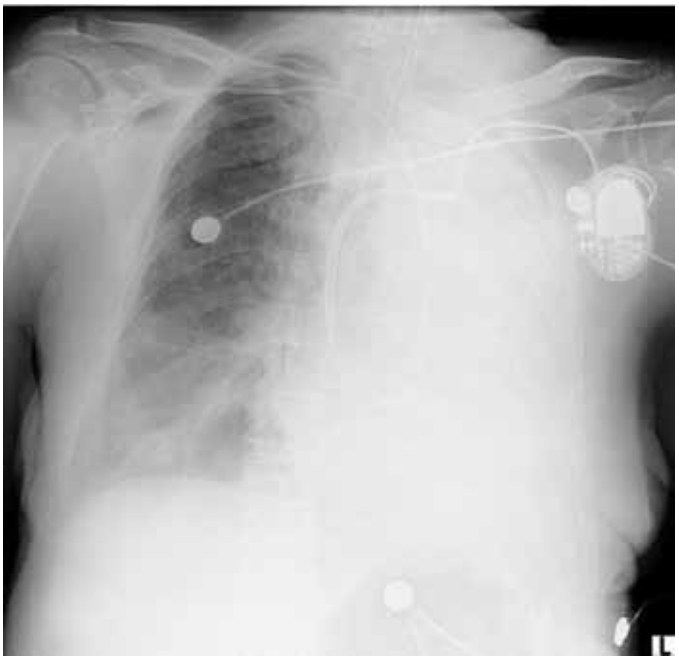

Figure 12: Misplaced central venous line. A central venous line was mistakenly installed into the right subclavian artery instead of the intended right subclavian vein, resulting in its projection into the ascending aorta. An endotracheal tube was also misplaced, with its tip positioned in the right main bronchus, resulting in atelectasis of the left lung. However, a nasogastric tube is correctly positioned. Furthermore, a dual-chamber cardiac pacemaker is in situ, with the appropriate positioning of both atrial and ventricular lead tips.

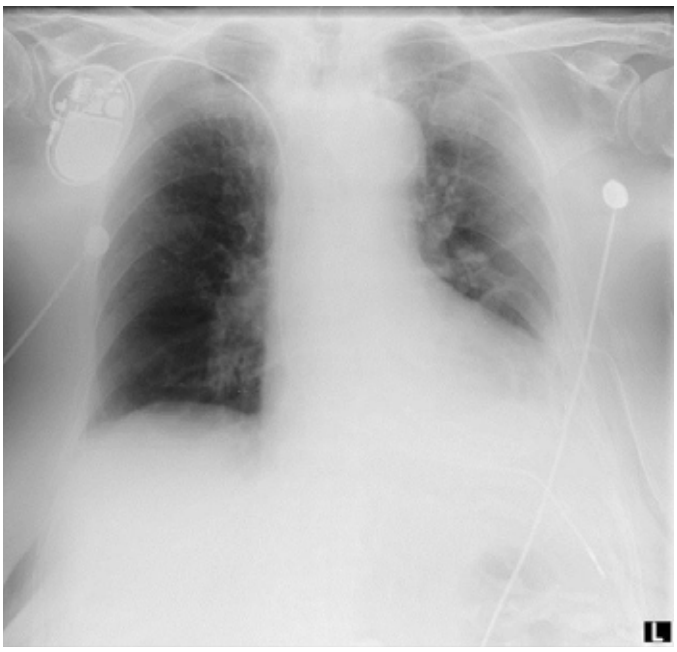

Figure 13: Misplaced pacemaker electrical lead. A single-chamber cardiac pacemaker is present, with the ventricular lead tip improperly positioned outside the cardiac silhouette, notably displaced into the left costophrenic angle. Cardiomegaly is evident, indicative of heart failure. Additionally, there is a left pleural effusion, for which a chest tube has been correctly inserted.

Diaphragmatic hernia

A diaphragmatic hernia is a defect in the diaphragm that causes abdominal organs to be displaced into the thoracic cavity. These hernias can occur as either congenital anomalies or acquired defects in the diaphragm. In the context of ICU chest radiographs, undetected diaphragmatic hernias may present as an unexpected finding in trauma patients, typically indicating an acquired type of hernia. Conversely, they can also be identified postnatally in newborns experiencing respiratory distress, often indicating an undiagnosed congenital diaphragmatic hernia (Figure 14). Regardless of its origin, a diaphragmatic hernia represents a life-threatening anomaly that should not be overlooked or misdiagnosed. Prompt identification and appropriate management are crucial for reducing associated risks and ensuring optimal patient outcomes [19].

Figure 14: Congenital left-sided diaphragmatic hernia. In a babygram, gas-filled bowel loops are observed projecting into the left thorax, accompanied by contralateral displacement of the heart and decreased lung ventilation. Additionally, a nasogastric tube is present, but its tip is positioned above the anatomically expected esophagogastric junction, raising suspicion of intrathoracic displacement of the stomach. The endotracheal tube is correctly positioned.

Conclusion

Chest radiography remains the preferred imaging modality for diagnosing cardiopulmonary disorders in ICU patients due to its accessibility, rapidity, and cost-effectiveness compared to other imaging techniques. Swiftly identifying emergency findings is a significant challenge that necessitates thorough training. Ultimately, the paramount objective is to save patients' lives, a task achievable through extensive knowledge and rigorous practice.

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Acknowledgments: None.

Funding: None.

Ethics Committee: All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This is an observational study. Formal consent is not required for this type of study because it contains no personal data and poses no concern regarding identifying information.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangement, etc.) that might pose a conflict of interest in connection with the submitted article.

Authors' Contributions: All authors contributed to the conception and design of the study. Material preparation, data collection, and analysis were performed by T Mandić Paulić, N Coce, L Filipović-Grčić, N Tičinović, and M Prutki. Tinamarel Mandić Paulić wrote the first draft of the manuscript, and all authors provided comments on previous versions. All authors read and approved the final manuscript.

Comet Tail Artifacts in Gray Scale Ultrasound and Colour Doppler Ultrasound in Various Anatomical Regions and Their Clinical Significance

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This article was submitted to RAD CASA - Medical Sciences as the review article

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 2 May 2025
Accepted: 29 May 2025
Published: 25 June 2025

Citation:
Ribić M, Coce N, Filipović-Grčić L, Ribić A, Planinić M, Čavka M. Comet tail artifacts in gray scale ultrasound and colour doppler ultrasound in various anatomical regions and their clinical significance

567=70-71 (2025): 102-108
DOI: 10.21857/94kl4c1zzm

Abstract

Artifacts are very common in ultrasound diagnostics. An artifact refers to any occurrence on the image that does not accurately represent the actual anatomical structures. Radiologists and doctors using ultrasound diagnostics should be able to recognize artifacts that have clinical significance in order to properly interpret ultrasound images. There are numerous examples in everyday clinical practice, which will be presented in this paper, where knowledge of the “comet tail” artifact in greyscale ultrasound and the “twinkling” artifact when using color Doppler can assist in clinical dilemmas.

Keywords: Ultrasound artifacts, Comet tail artifact, Twinkling artifact, Reverberation artifact, Greyscale ultrasound, Clinical significance, Differential diagnosis

Sažetak:

Artefakti repa kometa na klasičnom ultrazvuku i obojenom Dopplеru u različitim anatomskim regijama i njihova klinička važnost

Artefakti su uobičajena pojava u ultrazvučnoj dijagnostici. Artefakt predstavja bilo koju pojavu na slici koja ne predstavlja stvarne anatomske strukture. Radiolozi i ostali doktori koji upotrebljavaju ultrazvuk trebali bi moći prepoznati artefakte koji posjeduju kliničku važnost kako bi ispravno mogli protumačiti ultrazvučne snimke. Brojni primjeri iz svakodnevne prakse predstvaljeni u ovom radu pokazat će kako spoznaje o artefktima repa kometa u klasičnom ultrazvuku i svetlucajućem (twinkling) artefaktu na obojenom Doppleru mogu pomoći pri rješavanju kliničkih nedoumica.

Ključne riječi: ultrazvučni artefakti, artefakt repa kometa, artefakt svjetlucanja, reverberacijski artefakt, klasični ultrazvuk, diferencijalna dijagnoza

Figure 1: Haley comet, Edward Emerson Barnard, Yerkes Observatory, Wisconsin, 1910. Public domain, source: The New York Times, published July 3, 1910. Available on Wikipedia.

Introduction

The creation of an ultrasound image is based on the physical properties of the ultrasound wave, the passage of sound through tissue, the interaction of sound with reflecting obstacles, and the detection and processing of reflected waves. In radiological imaging, the term artifact is used for any occurrence on the image that does not faithfully represent the actual anatomical or pathological structures. [1] The "comet tail" artifact in greyscale ultrasound and the color comet tail or "twinkling" artifact when using color Doppler are subtypes of the "reverberation" artifact and they have appearance of comet on night sky. (Fig.1) [1,2] Therefore, artifacts can help us in clinical practice with many clinical dilemmas in ultrasound diagnostics. [1-4]
Small calculi in the bile ducts with minimal or no ductal dilatation, small kidney or ureteral stones with or without dilatation of the ductal system, calcifications of the ducts or parenchyma of the pancreas (in chronic pancreatitis), small calculi in intrahepatic bile ducts (in patients with cystic fibrosis), adenomyomatosis, gallbladder sludge (Fig.2), surgical clips, and foreign materials are some of the conditions where knowledge of the „comet tail" and „twinkling" artifact can lead to better detection and interpretation of underlying pathologies and therefore can benefit in everyday ultrasound practice. [2-4]

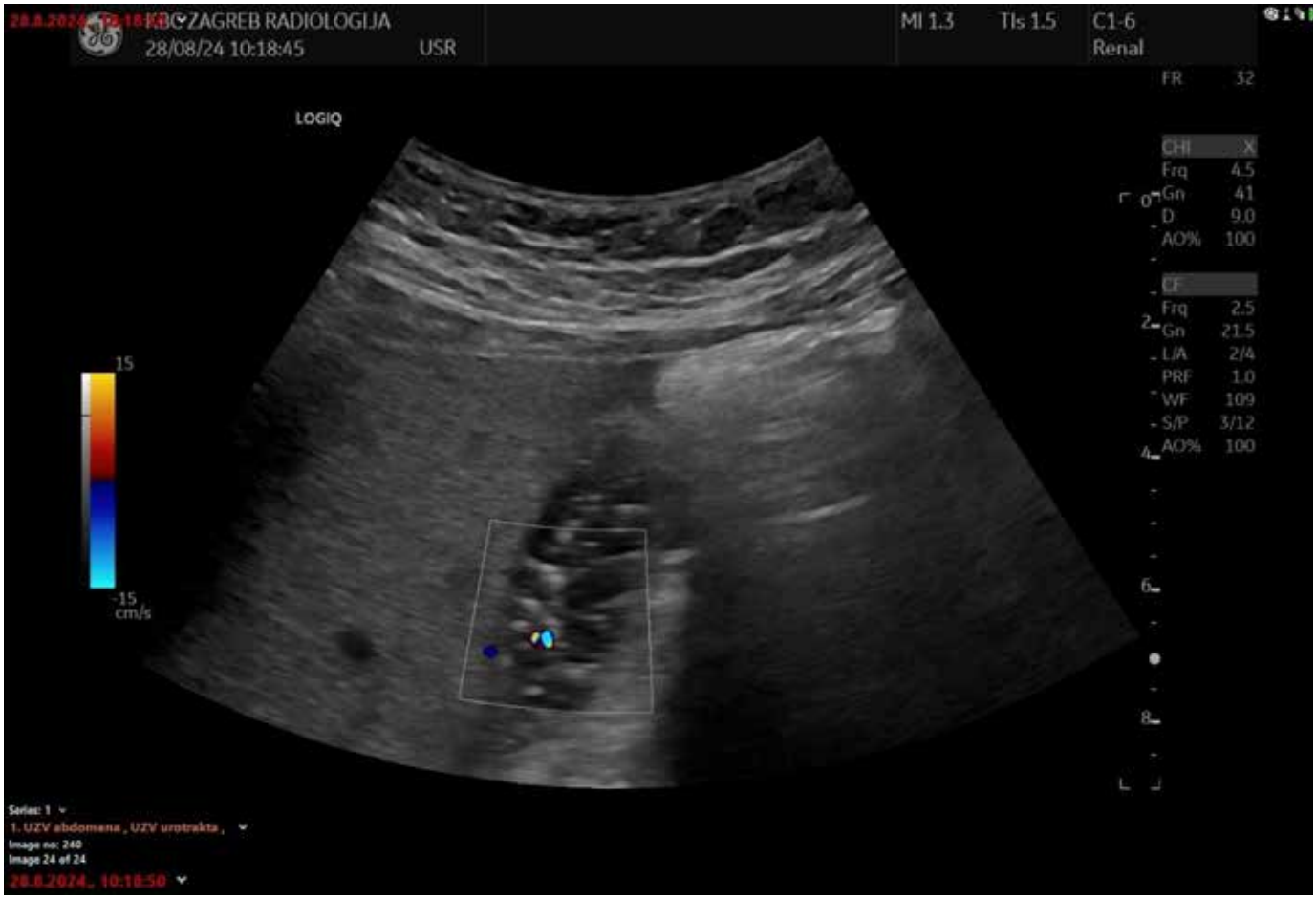

Figure 2 : Color comet tail or„Twinkling" artifact in gallbladder sludge and small calculi

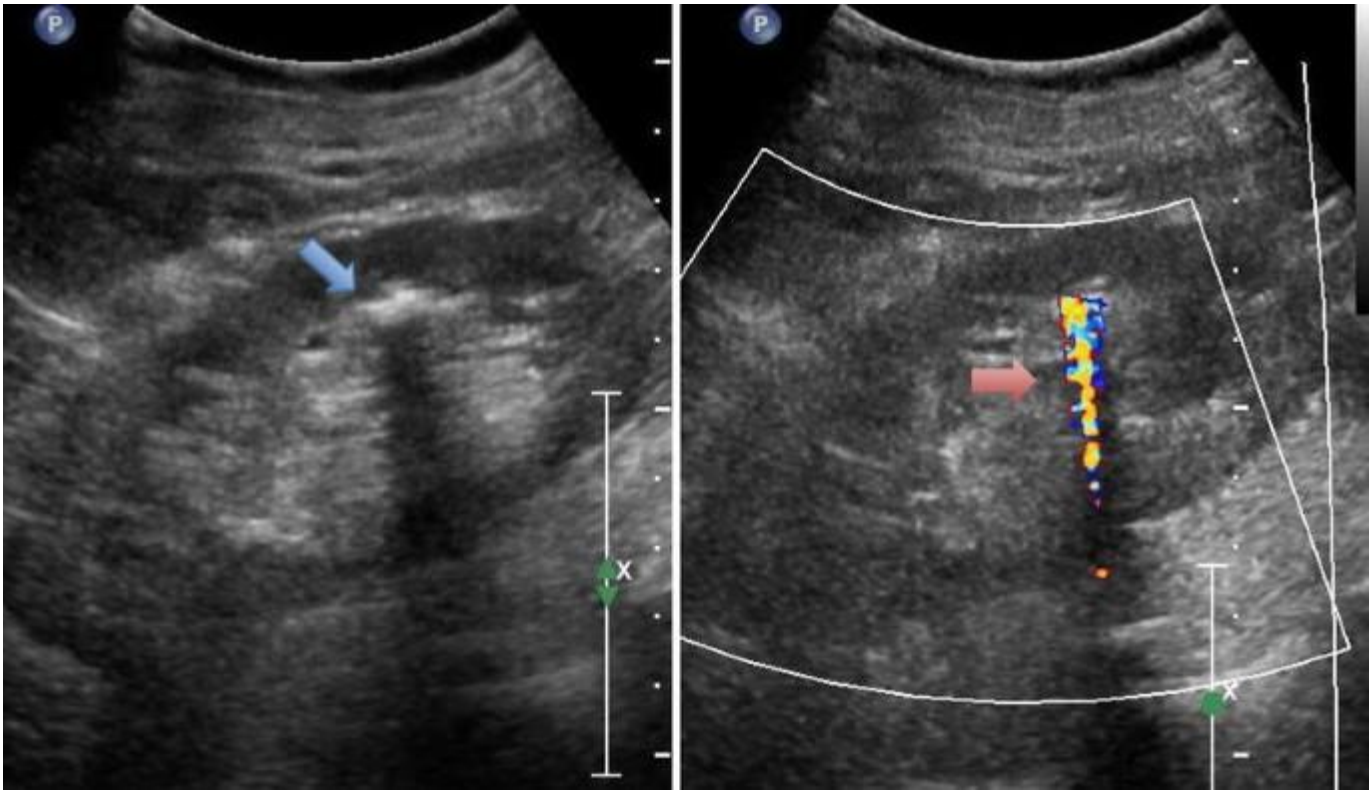

Figure 3: Color comet tail arterfact or „twinkling" aeterfact in small renal calculus

Figure 4: Comet tail artifact in the thyroid coloid cyst

Discussion

The ultrasound image is created based on several assumptions: every received reflected wave originates in the ultrasound probe itself, the reflected wave returns to the probe after a single reflection, the depth of the object from which the wave is reflected is proportional to the travel time of the emitted wave, the speed in tissue is constant, the path of the wave is straight, and the acoustic energy is uniformly attenuated through the tissue. [1,2,4] Such assumptions hold in ideal conditions, but in real clinical ultrasound practice, the received ultrasound waves are often displayed as artifacts. [1]

In radiological imaging, the term "artifact" is used for any occurrence on the image that does not faithfully represent the actual anatomical or pathological structures. In ultrasound diagnostics, artifacts can cause some structures to appear that do not actually exist. Conversely, some structures that do exist may not be displayed on the image, or the displayed structures may not be of the actual size, location, or appropriate brightness. It is important to note that the ultrasound technique itself is prone to artifact creation and therefore has the potential to interfere with the interpretation of findings. Therefore, radiologists and other physicians who use ultrasound as a diagnostic method should be able to recognize artifacts in order to properly interpret ultrasound images. [1]

The "comet tail" artifact in greyscale diagnostics and the color comet tail or „twinkling" artifact when using color Doppler are subtypes of the "reverberation" artifact. This means that parallel, closely positioned surfaces that reflect the ultrasound wave emitted from the probe cause the waves to bounce between themselves, resulting in the return of signals to the probe, which is "perceived" as a signal coming from deeper structures. [1,2,5] This occurs because there is a time delay from the emitted signal, which is displayed on the screen as an echogenic echo "posteriorly." In the case of the "comet tail" artifact, the reflecting surfaces are extremely close together and are not distinguished by the system, and in addition, their amplitude is reduced due to attenuation, so they appear as reduced in width. This effect on the screen or ultrasound image is seen as a triangularly narrowed shape described as the „comet tail" sign resembling the comet in the night sky (Fig.1). [1,5] If we use color Doppler, this will be displayed as turbulent flow. [2-4]

The formation of the artifact is primarily influenced by the hardness of the object being observed, but the settings during the ultrasound examination also play an important role. The artifact is sometimes more pronounced when increasing the color gain, moving the focus deeper than the object or when using a lower frequency probe. For example, with a high filter and high pulse frequency, the visualization of the resulting artifact increases. It is not fully clarified whether this occurs due to the enhancement of the artifact or simply better visualization, as the artifact Doppler shift is much greater than the actual Doppler shift of blood flow.

The „comet tail" artifact and the „twinkling" artifact can help us in everyday clinical practice, as seen through examples in further text. [3,4]

In the finding of cholelithiasis or choledocholithiasis, stones within the gallbladder are relatively clear when using B-mode, but sometimes when color Doppler is used, the artifact can help differentiate a certain formation from the artifact posterior to the lithiasis or, for example, in distinguishing a polyp from adenomyomatosis in which the artifact appears. Ultrasound shows focal or diffuse thickening of the wall, small anechoic cystic spaces of the affected segment of the gallbladder wall (Rokitansky-Aschoff sinus), and intramural echogenic foci with or without associated dorsal shadowing or reverberation artifact. As previously mentioned, cholesterol deposits and calcifications themselves cause the comet tail artifact, and when using color Doppler, the twinkling artifact. [1-3]

Sometimes it is difficult to visualize small calculi within the common bile duct with or without ductal dilatation; in such cases, the artifact is extremely useful. For example, in clinical suspicion of the presence of calcifications in intrahepatic bile ducts in patients with cystic fibrosis, the artifact can help if calcifications in intrahepatic bile ducts are not clearly seen in greyscale ultrasound. The next application or benefit of the artifact is found in chronic pancreatitis, where the ultrasound finding of extensive calcifications does not pose a significant problem in diagnosis using greyscale ultrasound. However, if the parenchyma is diffusely hyperechoic, for example, in fatty infiltration of the pancreas, it is then difficult to display small microcalcifications, in which case the display of the artifact serves as a method of differentiating existing microcalcifications. Similarly, the artifact can help in the diagnosis of splenic calcifications, for example, in granulomatous diseases. [3]

In the diagnosis of nephrolithiasis and urolithiasis, due to the nature and echogenicity of the background in the renal sinuses, small mineral concretions are sometimes almost impossible to visualize (Fig.3). Early renal nephrocalcinosis (Anderson-Carr kidney) is sometimes difficult to differentiate from hyperechoic fat of the renal sinuses themselves, and the artifact effect visible in nephrocalcinosis is then very helpful.Similarly, cortical nephrocalcinosis can differentially appear as hyperechoic renal parenchyma in diffuse parenchymal kidney disease. Another useful example of the artifact is when the presence of a stone in the middle and distal third of the ureter without dilatation is difficult to visualize due to the presence of air in the intestines or surrounding echogenic echoes of soft tissue. [3,4] Also, in polycystic kidney disease, linear calcifications along the wall may appear as hyperechoic echoes of the wall, without clear presence of calcifications, in which case the presence of the artifact helps in the final differentiation of what is displayed on ultrasound. [4]

In vascular ultrasound diagnostics, the clinical significance of the artifact can be applied in the finding of vascular wall calcifica-

tions and calcifications of the aneurysm wall and in the measurement of lumen diameter, where the artifact confirms the presence of calcifications.

There are also examples when the displayed ultrasound images do not show artifacts, for example, in the presence of intratesticular and extratesticular calcifications. The absence of the artifact is probably explained by the small surface area of the calcifications and the use of high-frequency probes. [3]

On the other hand, there are cases when it is not desirable to use the effect of the artifact because it can lead to incorrect conclusions. [3,5] For example, coarse thyroid calcifications very often cause the artifact, while punctate ones rarely do, so it is not advisable to use it for differentiation of punctate colloid calcifications in thyroid ultrasound, because in rare cases malignant microcalcifications may show signs of the comet tail artifact [3]. Some studies cited in the work of Van Trung Hoang et al. show that the comet tail artifact is typically found within or on the periphery of the follicle, while malignant microcalcifications are often scattered within solid tissue. Therefore, nodules with a large comet tail artifact that are within a cystic nodule are almost always benign (Fig.4). Other ultrasound characteristics of the lesion, such as taller-than-wide nodules, solid structure, or markedly hypoechoic and other specific characteristics, are more suspicious for malignancy. [5]

Hosokawa et al. described in their work the proven ultrasound presence of air in the portal vein, where by tracking hyperechoic foci distally, they identified the origin of the focus, i.e., the air bubble, which indicated intestinal pneumatosis of the stomach wall, sigmoid colon, and rectum. Intestinal pneumatosis can be the first sign of necrotizing enterocolitis, with air as a byproduct of bacterial metabolism entering the bloodstream. By tracking the origin of the gas bubble, we can detect the cause. [6] Since it is sometimes difficult to evaluate air in the intestinal wall due to motility and dorsal shadowing of air within the intestines, a clinically significant alternative can be tracking air in the branches of the portal vein to the intestinal wall. This is an example where differentiation of air in the lumen of the portal vein with the help of the artifact can be extremely important in making the correct diagnosis. [1,6] .

Conclusion

Ultrasound artifacts are an inherent part of diagnostic imaging and play a dual role: while they can sometimes obscure or distort anatomical structures, their recognition and understanding can also provide valuable clinical information. [1-6] The “comet tail” and color comet tail or “twinkling” artifacts, both subtypes of reverberation artifacts, are particularly significant in differentiating benign from some malignant lesions, identifying small calculi, and aiding in the diagnosis of various conditions such as gallbladder diseases, nephrolithiasis, and vascular calcifications. [2-4]

Recognizing these artifacts allows clinicians to avoid misinterpretation, reduce unnecessary additional testing, and improve diagnostic accuracy. [1,3] However, artifacts can also lead to diagnostic pitfalls if not properly identified, emphasizing the need for a thorough understanding of their physical mechanisms and clinical implications. [1,3,4] Ultimately, the ability to distinguish and appropriately utilize ultrasound artifacts enhances the utility of ultrasonography as a reliable and efficient diagnostic tool in everyday clinical practice. [1-6]

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Acknowledgments: None.
Funding: None.
Ethics Committee: All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This is an observational study. Formal consent is not required for this type of study because it contains no personal data and poses no concern regarding identifying information.
Conflict of Interest: Each author declares that he or she has no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangement, etc.) that might pose a conflict of interest in connection with the submitted article. Authors' Contributions: All authors contributed to the conception and design of the study. Material preparation, data collection, and analysis were performed by M Ribić, N Coce, L Filipović-Grčić, A Ribić, M Planinić and M Čavka. Matija Ribić wrote the first draft of the manuscript, and all authors provided comments on previous versions. All authors read and approved the final manuscript.

DOI: 10.21857/ypn4oc4189

Symposium: "Newer Insights in Brain Research"

Dear colleagues, participants, and esteemed guests,
it is our privilege to present the proceedings of the 24th Brain Awareness Week in Croatia, which took place from March 10th to 16th, 2025. As part of the international Brain Awareness Week initiative, this event continues to serve as a vital platform for the dissemination of current scientific knowledge on brain structure, function, and pathology, as well as for fostering interdisciplinary dialogue within the neuroscience community.

The 2025 programme was organised by the Croatian Institute for Brain Research (CIBR), University of Zagreb School of Medicine, in collaboration with the Croatian Society for Neuroscience (HDN), the Federation of European Neuroscience Societies (FENS), and as partners of the European Dana Alliance for the Brain (EDAB). Our efforts were further supported by the University of Zagreb School of Medicine, the Student Section for Neuroscience, the Student Section for Neurobiology of the Biology Students Association (BIUS, PMF), and numerous academic and clinical partners throughout Croatia. We gratefully acknowledge the Croatian Academy of Sciences and Arts (HAZU) for their continued support.

This year's scientific focus addressed three pressing topics:

- Neurodevelopment in the context of the digital environment and its implications for the child's brain
- Emerging challenges in neuroethics, particularly as neuroscience interfaces with technology and society
- Neuroplasticity and adaptive mechanisms in the brains of deaf and blind individuals

The programme comprised a series of scientific workshops, public lectures, and the central symposium, "Recent Advances in Brain Research," held on March 13th, 2025, at the HAZU Library Hall in Zagreb. These activities were designed to encourage critical discussion, promote evidence-based understanding, and bridge the gap between neuroscience research and public health awareness.

We extend our sincere gratitude to all contributors, presenters, and attendees whose engagement and expertise have enriched this event. We trust that the abstracts and discussions presented herein will stimulate further research, collaboration, and innovation in the neurosciences.

With best regards,
Ivica Kostović

Prenatal development of synapses and modulatory systems in the cerebral cortex of the human fetal brain

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Abstract:
New data on structure, function and modulation of synapses are related to mechanisms of presynaptic vesicle fusion (Sudhof and Malenka, 2008), dynamic nature of synapse plasticity, trans-synaptic interactions, nanoscopic organization of synapse structure (Yang and Annaert, 2021), evolution of neuromodulation (Galvin et al. 2018) and specificity of cortical synapses in humans (Christopoulou and Charrier 2024) in terms of assembly of postsynaptic scaffold via post-translational regulation of postsynaptic proteins (several hundred!) and finally, prolonged synaptogenesis, with specific tempo and maturation during life.
In this presentation I am focused on development and distribution of synapses during prenatal development of the human cortex. The results confirm (Kostovic 2024) that typical chemical synapses emerge during early fetal life and show consistent laminar compartmental relationship with distribution of distal dendrites of pyramidal neurons and transient neurons of the subplate and marginal zone. Contrary to the prediction of experimental investigators, typical electrical gap junctions were not found during prenatal synaptogenesis. It was also found that early presynaptic elements belong to the evolutionary new, advanced cholinergic systems, which make synaptic input together with thalamic axons. Evolutionary conserved monoaminergic axons arrive in synaptic strata during the same period and grow in cerebral anlage even earlier, that is during embryonic period. Within the synaptic strata monoaminergic axons may release transmitters non-synaptically. These new results are in accordance wit the concept of protracted period and specific tempo of life-long cortical synaptogenesis in humans. We conclude that recently discovered specifically human genetic regulation of presynaptic and postsynaptic molecular structure is already present during the early human fetal life.

Keywords: synapse, prenatal cortical development, presynaptic elements, synaptic compartments

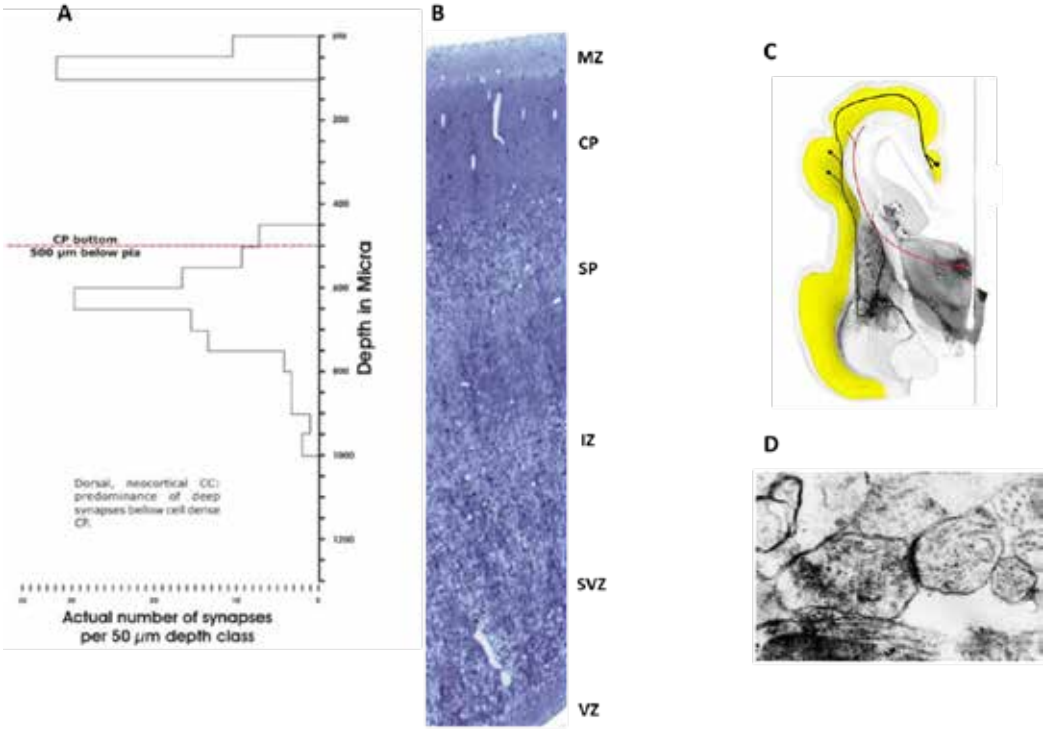

Figure 1. Distribution of synapses in neocortical part of gyrus cinguli in human fetal brain at 15 PCW. A- number of synapses per 50 µm depth class, B- Nissl stained 1 µm section adjacent to ultrathin section used for electron microscopy quanrification. Note that synapses are distributed in synaptic strata, C- potential afferent fibers to synaptic strata from cholinergic basal forebrain (Nucleus basalis Meynert), D- Typical asymmetric synapse at axonal terminal bouton and small dendritic profile in the SP.

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**This work was supported by the Croatian Science Foundation under the project number* IP-2024-05-7157

Concept Neurons

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Abstract:
Evidence from animal studies and the famous case of patient H.M. have convincingly demonstrated the crucial role played by the medial temporal lobe (MTL) in the formation, consolidation and retrieval of declarative memories. While many aspects of neural coding and retrieval of mnemonic information have been extensively studied, the specific neuronal mechanisms responsible for transforming our perceptions into long-lasting memories remain largely unknown. Recently, intracranial recordings in patients suffering from intractable epilepsy have shown that neurons in the MTL can be selectively activated when individuals are presented with stimuli corresponding to a specific concept, irrespective of the sensory modality through which the information is received.

For example, a single neuron recorded in the human hippocampus showed incredible specificity in its response to images of the actress Jennifer Aniston. This neuron was found to fire in response to different visual representations of the actress, as well as to a semantic representation of the same concept, such as the letter string "JENNIFER ANISTON". Furthermore, these neurons can activate when stimulus information is provided in other sensory modalities, such as when a specific name is spoken out loud. Due to this specificity, these neurons were initially named "*Jennifer Aniston neurons*". More recently, they have been referred to as "*concept cells*", reflecting their broader role in encoding abstract representations of semantic knowledge.

Given the well-established involvement of the MTL in the acquisition of declarative memory, one can conclude that concept cells serve as fundamental units for encoding the meaning of a stimulus for memory functions. Moreover, the way human memory tends to retain abstract concepts while discarding irrelevant details correlates well with the information encoded by the concept cells. The research has shown that these neurons sometimes fire to associated concepts, which are represented by different cell assemblies. When two concepts share a meaningful relationship, a subset of the neurons encoding one concept may also fire in response to another, giving a potential neural substrate for associative learning and offering a mechanistic explanation for how individuals transition from one concept to another in a fluid manner (Figure 1).

The described neural mechanism could serve as the neurobiological basis for episodic memory and even the stream of consciousness that characterizes human cognition. By linking perception with memory, concept cells generate an abstract and sparse representation of semantic knowledge that constitutes the building blocks for declarative memory functions, enabling humans to organize and recall information with remarkable efficiency (Quiroga 2012). Furthermore, recent studies have revealed that working memory representations are converted into long-term memory when concept cells become active.

Some researchers speculate that concept cells may represent a key component of human intelligence, potentially differentiating our cognitive abilities from those of other species. Future research on concept neurons could explore their precise role in the transition from perception to memory by investigating how their activity changes during different stages of learning and recall.

Keywords: temporal lobe epilepsy, depth electrode, declarative memory, visual perception, working memory

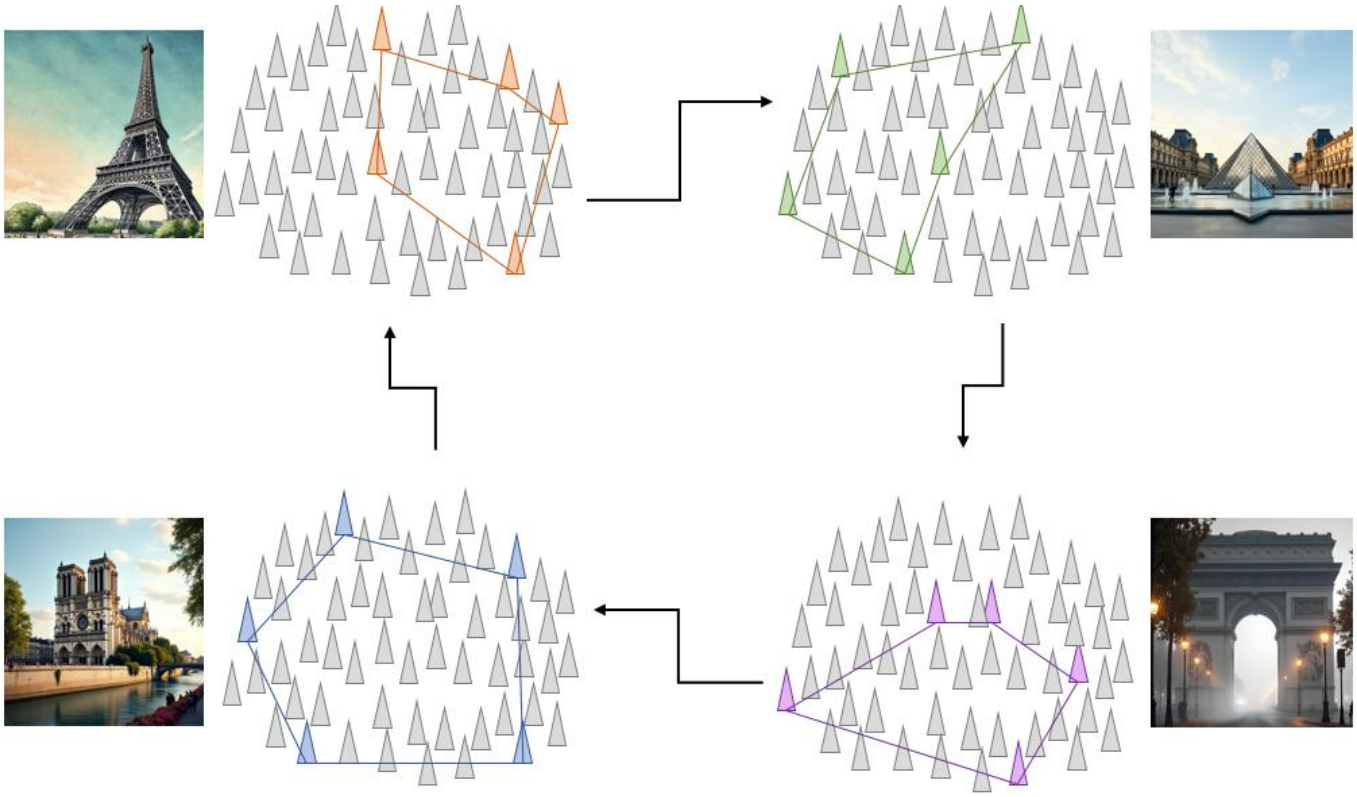

Figure 1. **Representation of the concept neurons in the medial temporal lobe.** *The encoded concepts of the representative Paris monuments are stored within a specific cell assembly. The top left (orange) assembly is activated upon recreating the concept of the Eiffel Tower, and at least one of these neurons is also included in the cell assembly that stores the concept of the Louvre museum shown in the top right (green), creating a potential mechanism of associative learning. The concepts of the Arc de Triomphe encoded by the cellular assembly in the bottom right (purple) and the Notre Dame of Paris cathedral stored by the assembly in the bottom left (blue) do not share a directly shared neuron but could be associated through the concept neurons encoding the concepts of the Eiffel Tower or the Louvre museum, existing within the same episodic memory (Quiroga 2012).*

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Extended developmental plasticity and vulnerability of human prefrontal microcircuits

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Abstract:

The transition from childhood to adulthood has become increasingly prolonged, with milestones such as completing education, marriage, and parenthood occurring later than ever before. This shift raises the question of whether adolescence should still be defined as the period between ages 12 and 18 or if its endpoint should be extended. Neurobiological research revealed that synaptic overproduction and developmental remodeling, including substantial elimination of synaptic spines, continue deeply into the third decade of life before stabilizing at adult level (Petanjek et al. 2023).

A recent study performing cross-species proteomic mapping of synapse development, tracked changes in over 1,000 postsynaptic density proteins from midgestation to young adulthood comparing human, macaque and mouse neocortex (Wang et al. 2023.). The results showed that human postsynaptic densities develop two to three times more slowly than those of other species, largely due to a higher abundance of RhoGEF proteins in the turquoise module. The turquoise module is linked to synaptic plasticity, human cognitive function and neuropsychiatric disorders. Overexpression of RhoGEFs was found to increase spine density and delay synapse maturation.

Several studies have previously demonstrated that gene expression changes during postnatal brain development in the human prefrontal cortex occur significantly later than in chimpanzees and rhesus macaques (Petanjek et al. 2023). The microcircuits responsible for processing the higher cognitive functions have the most extended period of synaptic overproduction, supporting high plasticity, which is crucial for acquiring complex cognitive abilities, including affective modulation of emotional cues, self-conceptualization, mentalization, cognitive flexibility, working memory and social skills. However, this prolonged development also extends the window of vulnerability, potentially increasing susceptibility to factors that may disrupt the formation of neural circuits involved in higher cognitive functions, which are impaired in neuropsychiatric disorders such as autism and schizophrenia (Petanjek et al. 2023).

A recent study performing single-nucleus RNA sequencing (Batiuk et al. 2022), found a reduction in GABAergic neurons and a concomitant increase in principal neurons within dorsolateral prefrontal cortex of patients with schizophrenia. The most pronounced changes were observed in the upper cortical layers, suggesting selective vulnerability and general cortico-cortical network impairment as a core substrate associated with schizophrenia symptomatology.

The dopamine system, whose disruption is considered to be one of the key features in schizophrenia, undergoes delayed maturation and changes throughout the whole stage of adolescence. Dopamine maturation interacts with changes in endocannabinoid signaling (Peters and Naneix 2022), characterized by transient increases in receptors expression and gradual rise in neurotransmitter levels. These changes enhance the recruitment of GABAergic interneurons and sustain the activity of pyramidal

neurons. Prefrontal dopamine signaling refines processing by improving the selection of specific inputs, decreasing the signal-to-noise ratio, most likely through regulation of glutamatergic synaptic spine pruning. Similar adolescent remodeling hasn't been observed in other neuromodulatory systems. Given their interactions with different neuronal populations and effects at different synaptic sites, dopamine and endocannabinoid signaling play a critical role in the late maturation of prefrontal circuits and their functioning.

Extensive reports showed that external factors, including drug use, nutritional habits, and stress, affect the functioning of the prefrontal cortex during adolescence. Research strongly indicates that these factors interfere with the development of dopamine and endocannabinoid pathways, ultimately impacting the reorganization of cortical microcircuits. A recent epidemiological study conducted on a large Danish cohort found an association between cannabis use disorders and the onset of schizophrenia (Hjorthøj et al. 2023). The study indicated that 15-30% of schizophrenia cases in males, and 5-10% in females could potentially be prevented if cannabis use disorders were avoided between ages 15 and 25. This provides a very explicit example of how the adolescent and post-adolescent brain remains extremely plastic and vulnerable to external factors.

Recognizing the extended developmental window in humans is crucial, as it entails both increased plasticity and vulnerability of prefrontal microcircuits. The influence of environmental factors on the shaping of these microcircuits is significant and may contribute to the development of late-onset neuropsychiatric disorders. Insights into prolonged neurodevelopment should be carefully considered when designing psychological, social, and educational strategies for adolescents and young adults, with a particular focus on legislation affecting those under 25.

This work was supported by the Croatian Science Foundation under the project number HRZZ-IP-10-2022-8943 (Uniqueness in development of interneurons in human prefrontal cortex during fetal life and first postnatal year – implications in pathogenesis of schizophrenia and autism)

Keywords: prefrontal cortex, dendritic spine, glutamate, dopamine, endocannabinoids

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Figure 1: High-power magnification images of rapid Golgi-impregnated layer IIIc pyramidal neurons in the dorsolateral prefrontal cortex showing synaptic spines on dendritic segments receiving thalamic (left panel) and cortical input (right panel) across various developmental stages: a 1-month-old infant, a 2.5-year-old child, as well as subjects aged 16, 28, and 49 years. The highest synaptic spine density is observed at the age of 2.5 years and dendritic segments receiving cortical input do not show significant decline at the age of 16 years, indicating that protracted reorganization of associative cortico-cortical circuits extends into the third decade of life.

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Functional imaging of associative areas of the cerebral cortex

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Abstract:

Brain imaging using functional magnetic resonance imaging (fMRI) enables the study of functional connectivity between different brain regions at rest, when the individual is not performing a specific task. Resting-state functional connectivity analysis has become a key tool in neuroscientific research of psychological and neurological disorders. The ability to observe how different brain regions communicate, as well as how these connections can be disrupted in various disorders, opens new horizons for understanding, diagnosing, and potentially treating these conditions.

In Alzheimer's disease, reduced connectivity in the Default Mode Network (DMN) affects memory processes, while in Parkinson's disease, reduced connectivity in dopaminergic networks impairs motor functions. In autism, decreased connectivity between temporal and frontal regions explains difficulties in social interaction, and in ADHD, reduced connectivity between prefrontal regions and other areas of the brain is linked to problems with attention and impulsivity. In depression, reduced connectivity in networks for emotional regulation, such as prefrontal and limbic areas, may help in understanding the disorder. For anxiety disorders and PTSD, changes in the connectivity of the amygdala with prefrontal regions explain hyperreactivity to stress.

In patients with schizophrenia, it has been shown that the functional connectivity of the thalamus and the primary motor and somatosensory cortex is increased, while the functional connectivity of the thalamus and the prefrontal cortex, striatum and cerebellum is reduced (Murray and Antičević 2017.). Recent research shows that the functional architecture of neural networks can be used as a neuroradiological biomarker for the recognition of mental disorders including schizophrenia (Spronk et al. 2021). Although at this moment the sensitivity and specificity of fMRI-based diagnostics are not satisfactory for clinical use, it is likely that with the development of MRI protocols and post-processing analysis the problem will be solved in the future.

Recent advancements in neuroimaging have also provided valuable insights into the auditory language comprehension and intrinsic network abnormalities associated with Autism Spectrum Disorder (ASD). Hua et al. (2024) conducted an ALE meta-analysis of fMRI studies to investigate auditory language processing among children and adolescents with ASD, revealing altered brain activity in key regions involved in speech and language. Similarly, Goodwill et al. (2023) employed meta-analytic connectivity modeling to examine functional connectivity patterns in ASD, uncovering disruptions in neural circuits that underlie social and cognitive functioning. In another study, Yoon et al. (2024) utilized independent component analysis to explore intrinsic network abnormalities in children with ASD, providing evidence of atypical connectivity within brain networks implicated in sensory processing and executive functions. These studies provide deeper insight into the neural mechanisms underlying

language and social cognition in individuals with ASD, offering potential targets for future therapeutic interventions.
Ongoing research in this field promises to enhance our understanding of neurological disorders and pave the way for the development of targeted therapies based on functional brain networks.

Keywords: Autism Spectrum Disorder; functional MRI; Schizophrenia

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The role of the extracellular matrix in neurodevelopmental brain disorders: a genetic perspective

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Abstract:

The fetal cerebral wall is rich in extracellular matrix molecules (ECM) that fill the intercellular space, estimated to comprise up to 60% of the volume of the early fetal brain. Four numerous families of ECM molecules (hyaluronan, proteoglycans, glycoproteins, and link proteins) are the main components of the biochemical niches that mediate the distribution of key signaling and trophic factors during prenatal development and build mechanical scaffolds to conduct actively morphogenetic processes such as cell proliferation, migration, axon pathfinding, and cortical folding. The transient fetal zones, such as the marginal zone, subplate, and early intermediate zone, are rich in hydrated extracellular matrix (ECM), and the last two are the bases of the cortical (hyperintensity) pattern on MRI scans, highlighting the importance of ECM in brain diagnostic and prognostic MRI follow-up in fetuses. In collaboration with their receptor, the ECM components also play a significant role in the differentiation of dendrites and the genesis of spines and synapses. Finally, some ECM molecules become integral to the quadripartite synapse and perineuronal nets, contributing to the formation of the functional connectome. Humans have more ECM in the cortical wall during fetal development compared to the evolutionary closest primate species. This supports the ECM as an evolutionary force, in conjunction with prolonged brain development, which facilitates the formation of more synapses and more elaborate, complex connections, while also being an additional substrate for vulnerability and plasticity in humans. Mutation of ECM coding genes or dysregulations of the ECM molecules' expression spatial and temporal patterns, can affect the processes of cell proliferation, migration, cortical folding, or differentiation, leading to a heterogeneous group of disorders- known as malformations of cortical development (MCD, such as microcephalia, megalocephalia, lissencephaly, polymicrogyria, periventricular nodular or subcortical band heterotopia), or neurodevelopmental disorders (NDD), such as epilepsy, autism spectrum (AS), schizophrenia, and/or intellectual disabilities (ID). With the latest advances in sequencing technologies, greater accessibility to whole-genome and whole-exome sequencing (WGS, WES) for diagnostic purposes has led to an increase in the number of mutated genes coding for ECM components identified in patients and linked to MCD or NDD. A few examples include mutations in the heparan sulfate proteoglycan 2 (*HSPG2*) and laminin subunit gamma 1 (*LAMC1*) genes, which are associated with disrupted proliferation in the ventricular and subventricular zones, leading to microcephaly, hemimegalencephaly, or focal cortical dysplasia. The mutations in genes coding glycoprotein reelin, ECM glycosylation enzymes, O-mannosyltransferase enzyme, and glycosyltransferase-like protein, *or the adhesion G—protein-coupled receptor (RELN, POMT1, LARGE, and GPR56, respectively), have been found* to hinder migration and cell adhesion, causing lissencephaly or cobblestone lissencephaly, bilateral frontoparietal polymicrogyria, or ID. Proteoglycans, including

versican, neurocan, lumican, hyaluronan, and proteoglycan link protein-1, as well as collagens (encoded by *VCAN, NCAN, LUM, HAPLN1*, and *COL4A1*, respectively), are expressed in the subplate and marginal zone. When mutated or dysregulated, these proteins alter synaptogenesis and gyrification, resulting in MCD or ID. The genes *PAX6, TBR1, SOX2, and MEF2C* coding for transcription factors, as well as members of the Wnt and Notch signaling pathways, enzymes involved in DNA methylation and histone modifications, and miRNAs, regulate the quantity and distribution of the ECM and their dysregulation can be part of the pathogenesis of MCD and NDD. For example, the *NDST1 and CHSY1 genes, which encode enzymes involved in ECM biosynthesis, are found to be* mutated in patients with AS and IP (for additional examples, see Table 1). The new research paradigm, which leverages the potential of generating induced pluripotent stem cells from patients with MCD or NDD to cultivate cerebral organoids, is an exceptional tool for elucidating the function of ECM and its receptor genes in the developing brain, both in health and disease. Therefore, knowledge of the genetic basis of brain-specific ECM and its MRI representation contributes to understanding the pathogenesis of cortical malformations and brain function abnormalities, and has proven essential in identifying potential new targets for diagnostic and therapeutic approaches to treating neurodevelopmental disorders.

Keywords: subplate, synaptogenesis, malformation of cortical development, autism spectrum disorders, perineuronal nets

Acknowledgments: This work was supported by the Croatian Science Foundation (IP-2024-05-4135, CortProteG).

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Table 1 Overview of extracellural matrix (ECM) components and their genes associated with some malformations of cortical development (MCD) and neurodevelopmental disorders (NDD).

Table 1. Overview of extracellural matrix (ECM) components and their genes associated with some malformations of cortical development (MCD) and neurodevelopmental disorders (NDD).

MCD or NDD group	MCD type or NDD	Gene	ECM component
Disorders of neuronal and glial proliferation or apoptosis	Microcephaly	*HSPG2*	Perlecan
		GPC1	Glypican-1
		LAMC1	Laminin subunit gamma 1
	Hemimegacephaly	*HSPG2*	Perlecan
		LAMC1	Laminin subunit gamma 1
	Hydrocephaly	*COL1A1*	Collagen I alpha 1
		COL1A2	Collagen I alpha 1
	Focal cortical dysplasia (type II)	*TNC*	Tenascin-C
		TNR	Tenascin-R
		VCAN	Versican
		BCAN	Brevican
		MMP9	Matrix metalloproteinase 9
		PLAT	Tissue-Type Plasminogen Activator
		PLAU	Urokinase-Type Plasminogen Activator
		HSPG2	Perlecan
		LAMC1	Laminin subunit gamma 1
	Tuberous sclerosis complex (TSC; cortical tuber)	*LAM*	Laminins
		COL	Collagens
		TNC	Tenascin-C
		ITGB4	Integrin beta 4
		PLAT	Tissue-Type Plasminogen Activator
		PLAU	Urokinase-Type Plasminogen Activator
Disorders of neuronal migration	Lissencephaly	*RELN*	Reelin
	Lissencephaly-pachygyria	*COL18A1*	Collagen XVIII alpha 1
	Cobblestone malformations	*LAMB1*	Laminin subunit beta 1
		LAMB2	Laminin subunit beta 2
		LAMC1	Laminin subunit gamma 1
		POMT1	Protein O-mannosyl-transferase 1
		LARGE	Glycosyltransferase-like protein LARGE1
		GPR56	Adhesion G protein-coupled receptor G1
		RELN	Reelin
Disorders of post migrational development	Polymicrogyria (PMG)/ bilateral persylvian PMG	*SRPX2*	Sushi repeat-containing protein SRPX2
		LAMC3	Laminin subunit gamma 3
		RELN	Reelin
		POMT1	Protein O-mannosyl-transferase 1
		LARGE	Glycosyltransferase-like protein LARGE1
		GPR56	Adhesion G protein-coupled receptor G1

NDDs	Schizophrenia	*RELN*	Reelin
		CPSG	Chondrotin suphate proteoglycan
		ADAMTSL3	*A Disintegrin-like And Metalloprotease domain with ThromboSpondin type I motifs-Like 3 gene*
		SEMA3A	Semaphorin 3A
	Autism spectrum disorders	*MMP9*	Matrix metaloproteinase-9
		RELN	Reelin
		VCAN	Versican V2
		NDST1	N-deacetylase and N-sulfotransferase 1
		CHSY1	Chondroitin sulfate synthase 1
	Intellectual disabilities	*VCAN*	Versican
		NCAN	Neurocan
		LUM	Lumican
		HAPLN1	Hyaluronan and proteoglycan link protein 1
		COL4A1	Collagen IV alpha 1
		RELN	Reelin
		POMT1	Protein O-mannosyl-transferase 1
		LARGE	Glycosyltransferase-like protein LARGE1
		GPR56	Adhesion G protein-coupled receptor G1

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Human-specific genetics: new insights and research opportunities into the molecular basis of neurodevelopmental disorders

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Abstract:

Neurodevelopmental disorders (NDDs) encompass conditions that impair the development of the nervous system, affecting cognitive, emotional, and behavioral functions. Their complex etiology involves a combination of genetic, environmental, and neurobiological factors. Advances in genomics have identified various genetic alterations, including chromosomal anomalies, copy number variations, single-nucleotide polymorphisms, and rare *de novo* mutations, all of which are linked to NDD pathogenesis. However, despite a strong genetic basis, the causes of many NDD cases remain unknown. Current research investigates how genetic modifications influence human neurodevelopmental evolution, shaping our distinct cognitive abilities. NDDs primarily affect functions distinguishing humans from other species, such as abstract thinking. Given DNA's role as a cellular blueprint, one might assume a "more is better" paradigm in human genome complexity. However, genome size alone does not define cognitive ability; for instance, the fork fern *Tmesipteris oblanceolate* has a much larger genome than humans measuring 160.45 Gbp/1C. Instead, evolutionary comparisons with our closest relatives, primates, offer better insights. Humans and chimpanzees share approximately 96% of their genomes. Yet, key genomic rearrangements differentiate them, falling into four categories: regions that rapidly accumulate changes through evolution, regions that have been lost, duplicated regions, and regions with variable copy numbers in humans.

One striking example of genomic evolution is the duplication of the *ARHGAP11B* gene, which is linked to brain expansion. Expressed in ape cerebral organoids, *ARHGAP11B* increases the number of basal progenitors to human levels (Fischer et al. 2022).

Earlier research focused on gene gains, assuming evolutionary advantages stemmed from additional genetic material. However, recent studies highlight the role of gene loss. Human-specific deletions of conserved elements (hCONDELs), primarily located in noncoding regions, can impact gene expression. A study identified 10,032 hCONDELs, affecting intergenic (59%) and intronic (35%) regions, with 800 showing species-specific regulatory activity. Many deletions lead to transcriptional repression rather than activation. For example, a single-base deletion in *LOXL2* removes a repressor binding site, resulting in increased gene expression in humans compared to chimpanzees. Genome editing confirmed this by reintroducing the ancestral base, restoring chimpanzee-like expression levels. Single-cell RNA sequencing further assessed the impact of this human-specific deletion. Edited cells containing the ancestral sequence showed significantly lower *LOXL2* transcription, mirroring chimpanzee profiles. This regulatory change triggered widespread alterations in gene expression, with 145 genes exhibiting differential expressions. Gene ontology analysis highlighted processes related to cell migration, development, myelination, intercellular transport, and synaptic function (Xue et al. 2023)032 human-specific conserved deletions (hCONDELs.

Another key feature of genome evolution are human-accelerated regions (HARs), which accumulate changes at an unusually high rate. A well-studied example, HARE5, regulates *FZD8*, a receptor in the WNT pathway. HARE5 is expressed earlier and more intensely during human brain development than in chimpanzees, leading to increased neural progenitor proliferation and larger brain size in transgenic mice (McLean et al. 2011)"properties":{"formattedCitation":"(McLean et al. 2011. Initial hypotheses suggested that human-specific structural variants (hsSVs) might alter genome folding, leading to enhancer hijacking—a phenomenon in which conserved enhancers regulate new target genes. However, recent studies mapping HARs and human-gained enhancers (HGEs) in neural stem cells indicate that most HARs still regulate the same genes in both species, challenging this hypothesis (Pal et al. 2025).

Genomic regions with variable copy numbers also contribute to human-specific traits. One such gene, *SRGAP2C*, arose from a partial duplication of *SRGAP2A*. *SRGAP2C* inhibits *SRGAP2A*, enhancing synaptic density in cortical pyramidal neurons, improving cortico-cortical connectivity, refining neuronal responses, and strengthening cognitive functions in transgenic mice (Schmidt and Polleux 2022).

What are the implications for NDDs? Genetic variations in human-specific regions are linked to NDDs. Patients with diverse clinical manifestations, such as microcephaly or reduced gyrification, often exhibit mutations or deletions in genes unique to humans. Traditional sequencing methods initially identified these changes, but research now incorporates single-cell epigenomic and transcriptomic comparisons. Brain development involves dynamic gene regulation across diverse cell types, rendering human brain evolution a complex and intricate process. The extended developmental period, increased synaptic complexity, and enhanced connectivity of the human brain stem from genomic and epigenomic modifications. These insights enhance our understanding of both human cognitive evolution and NDD genetics, informing future research on how our unique genome influences brain function.

Keywords: neurodevelopmental disorders (NDDs), genomic evolution, human-specific genetic variations, human-accelerated regions (HARs)

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Imaging Mass Spectrometry – A Window into the Molecular Diversity of the Brain

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Abstract:

The molecular complexity of brain composition underlies its anatomical complexity. The extraction of molecules from tissue homogenates facilitates molecular analysis but results in a loss of anatomical organization. By employing histological and multiplex immunohistochemical methods, the histology of the tissue is preserved, but only a limited number of molecules (1 – 100) can be visualized. Adding to the limitations of traditional research, histological epitopes are often selected based on hypotheses that may prove incorrect, leading to longer and more expensive investigations.

To address these challenges, the method of imaging mass spectrometry (IMS) was developed. IMS maintains the anatomical integrity of the tissue while measuring over a thousand molecular signals. Depending on tissue processing, the resulting signals can reflect protein composition (proteomics) or pertain to small metabolites (metabolomics) and lipids (lipidomics). When similar samples of normal tissue are available, additional statistical analysis helps identify molecules with statistically significant deviations. Further bioinformatics processing, using existing databases, allows these molecules to be placed within relevant metabolic processes or to determine transcription factors influencing their levels.

Unlike studies that begin with a hypothesis, IMS is often hypothesis non-driven or untargeted analysis. Such analysis aims to eliminate researcher bias and identify relevant pathophysiological mechanisms. IMS can also be targeted, focusing on just one or a very small set of molecules. This approach can reveal the distribution of a drug and its metabolites in brain tissue, assess viral neurotropism, or illustrate the distribution of a toxic metabolite.

In our previous works, we explored both approaches. Using untargeted IMS analysis of the brains of mice with a knockout gene for GD3 synthase and a deficiency of two (GD1b and GT1b) of the four main gangliosides, we demonstrated a disruption in the synthesis of ubiquinone, porphyrin, and long-chain fatty acids. In the study presenting a new method for isolating lipid rafts without the use of detergents, targeted IMS was employed to demonstrate the loss of all four main glycolipids in the brain (GM1, GD1a, GD1b, and GT1b) from cerebellar tissue sections after treatment with detergents Triton X100 and Brij O20.

Whether targeted or untargeted, IMS analysis generate large datasets, accelerate research, and enhance the utility of rare samples. Combined with other imaging or biochemical methods, IMS is a powerful tool in translational medicine.

Keywords: imaging mass spectrometry, multiplex methods, gangliosides

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Acknowledgement:
This work has been funded by the Croatian Science Foundation grants HRZZ IP-2014-09-2324 and IP-2016-06-8636, European Union through the European Regional Development Fund, Operational Programme Competitiveness and Cohesion, grant no. KK.01.1.1.01.0007 (CoRE – Neuro) and grant no. KK.01.1.1.02.0015, and University of Zagreb research support grant NEURO-MOD-PUMP, 10106-24-1546.

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Abnormal orbits of genetic mechanisms in the formation of brain tumors

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Abstract:

In spite of recent progress, molecular mechanisms responsible for brain tumor formation are still inadequately explained. These are very complex mechanisms that include changes in genes and proteins involved in numerous vital cellular processes - proliferation, apoptosis, DNA repair, mobility, angiogenesis, immune surveillance, genomic instability and cellular metabolism. The famous hallmarks of cancer proposed by Hanahan and Weinberg in 2000. have been constantly upgraded and now number 14 characteristics. Latest additions include unlocking phenotypic plasticity, nonmutational epigenetic reprogramming, polymorphic microbiomes, and cellular senescence. However, signal transduction pathways are fundamental in the development and progression of cancer. The imbalances in cellular signaling networks are also causative of brain tumors. The recent failure of a number of targeted therapies, particularly for glioblastoma, shows that CNS tumors do not just respond to a single pathway-driven targeted therapy. On the contrary, targeting multiple pathways simultaneously could be an alternative way to overcome tumorigenesis. Our group is studying brain tumor genetics with particular focus on Wnt signaling. We believe that genetic and expression changes are associated to phenotypic characteristics and behavior of tumor cells. The results of in silico analysis by cBioPortal for Cancer Genomics (Figure 1) database following Array Comparative Genomic Hybridization (aCGH) and Genomic Identification of Significant Targets in Cancer (GISTIC) identified significantly deleted regions: 9p21.3; 17p13.2; 10q24.2; 14q21.3; 1p36.11 and 13q12.11, but also amplified ones: 3q28; 12q13.3 and 21q22.3 in higher malignancy grades of gliomas. We identified copy number aberration (CNA), pathways and genes that are biologically and functionally significant by the use of DAVID, an enrichment analysis tool designed to estimate the biological relevance of a given collection of genes, and data repository KEGG (Kyoto Encyclopedia of Genes and Genomes). It should be emphasized that other cellular mechanisms are also crucial for the formation of brain tumors, for example oncogenic roles of long non-coding RNAs in signaling regulation. Novel research focuses on the tumor microenvironment and glioma stem cells, the appearance of neoantigens and cellular senescence that creates a specific SASP phenotype (senescence-associated secretory phenotype) responsible for therapy resistance. All this contributes to a better understanding of the brain tumor genetic profile. The highlighted molecular changes can offer prognostic markers and directions for the development of improved treatment.

Keywords: genetic profiles of brain tumors, cBioPortal, aCGH, GISTIC

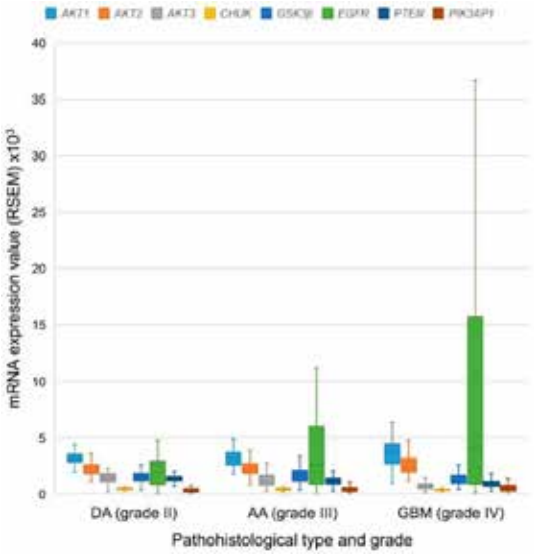

Figure 1. Distribution of mRNA expression of the examined genes according to the pathohistological type and grade of diffuse gliomas obtained by the RSEM method. DA—diffuse astrocytoma; AA—anaplastic astrocytoma; GBM—glioblastoma multiforme (Brlek et al, 2021).

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Cerebral Organoids enable studying live surrogates of brain tissue, patient/donor specific, in long term experiments *in vitro*

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Abstract:

Cerebral organoids (COs) are millimeter-sized, self-organizing 3-dimensional live tissue spheres derived from human induced pluripotent stem cells (iPSCs) differentiated into neural tissue. Standard COs represent mixed-regions of cortical tissue, but directed organoids represent specific cellular subtypes specific for some neuro-anatomical regions, such as dorsal forebrain cortex, hippocampus, striatum, medial gangliomic eminence, midbrain and cerebellum. Functional connectivity between different parts of the brain can be studied by deriving each regional organoid separately and then fusing the parts into assembloids. Organoids were initially thought as useful models of neurodevelopmental (such as microcephaly)[1] and related disorders (such as ZIKA-virus infections causing microcephaly), but not for conditions related to old-age neurodegeneration, due to embryonic nature of the starting cells, and due to erasing of epigenetic ageing marks by the iPSC re-programming. Recent studies however, including studies by Nizetic group in collaboration with Croatian Institute for Brain Research (CIBR), uncovered that cerebral organoids, most probably due to the lack of clearing mechanisms (such as microglia, blood-derived macrophages, glymphatics linked to circulation) surprisingly can accumulate aggregates causative of neurogenerative conditions[2,3]. We and others have uncovered that organoids can model the true causative sequence of events representing Alzheimer's disease (AD) pathology, and allow studies of the effects of gene dose and drugs on AD prevention, progression and spreading[2]. Studying patients born with an extra-copy of the gene for the Amyloid Precursor Protein (APP) that causes early onset Alzheimer's disease (EOAD), including both patients with the segmental duplication of this gene (Dup*APP*) and people with Down Syndrome ((DS), born with an extra copy of human chromosome 21, harbouring the *APP* gene), we uncovered that their cerebral organoids develop in just over 3 months in culture typical hallmarks of Alzheimer's disease (AD) pathology: secretion of toxic soluble oligomeric aggregates of β-amyloid peptide, phosphorylated Tau and inflammasome ASC-specks[3], extracellular amyloid insoluble fibrillary deposits, intracellular pathologically conformed Tau protein, and progressive neuronal loss[2]. We also uncovered that the profile of β-amyloid peptides secreted by the organoids to the culture media, closely recapitulates the profile visible in cerebrospinal fluid of people with DS[2]. Importantly, all 3 pathological processes can be reversed by the inhibition of the β-amyloid peptide, therefore recapitulating the true sequence of events in human AD: β-amyloid-peptide-driven Tau-opathy. This is also providing the proof-of-principle that the organoid technology can be used for drug-screening approaches to uncover compounds with potential to prevent or slow-down AD pathogenesis[2].

In other recent studies, we have shown that not only AD, but also cellular and neuronal ageing can be modelled using COs[4]. We revealed that premature ageing in DS is underpinned by cellular senescence that can be modelled in induced pluripotent stem cells (iPSCs) and cerebral organoids. We

also showed that DNA damage-associated progeria, with a decrease in LaminB1 levels, is a significant component of DS, that trisomy of the chromosome 21-encoded gene *DYRK1A* causes this, and that it can be pharmacologically alleviated in iPSCs and COs[4]. This study included a collaboration with 6 institutions in Zagreb and was partly funded by the Croatian Science Foundation. Through this study, a transfer of technology was achieved enabling the group at the Croatian Institute for Brain Research (CIBR) to adopt the iPSC and COs technologies. This is now further advanced, in collaboration with Nizetic lab in London, by producing assembloids between striatal and cortical organoids, for the mechanistic study of neuronal connectivity defects in DS.

Our studies demonstrate that COs, despite lacking many cell types and precise 3D tissue organization of the human brain, can model processes that mimic those in the human brain, including ageing, neuro-inflammation and neurodegeneration. Furthermore, lack of certain brain types in organoids is also an advantage, as it allows sequential addition of the missing cell types (such as astrocytes, microglia or endothelial cells derived from same, or genetically altered iPSCs), and thereby the precise dissection of the roles of each cell type in the disease being studied.

Organoid technology appears promising for the uncovering of new insights about the mechanisms, biomarkers of disease risk and progression of prognostic significance, as well as detecting new chemical compounds and antibodies for the developments of hitherto unknown therapeutic approaches[5].

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Changes in gene regulation in brain aging

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Abstract:

The greater the mortality caused by environmental factors, the less natural selection will favor the maintenance of somatic cells, giving preference to the vitality of germ cells. Since the pressures of natural selection act more strongly towards positive effects in earlier life stages (gene variants that lead to dysfunction before puberty will quickly be outcompeted by alleles that favor the production of healthy offspring), the aging process is a consequence of the absence of these pressures after the reproductive period. Cognitive abilities peak during the reproductive phase and need only remain stable until offspring become independent. Events after reproduction are less significant, so the heritability impact on longevity is generally low: approximately 0.23 for men and 0.26 for women (**Herskind et al., 1996**). Genetic effects on lifespan shorter than 60 years are minimal, and their impact then increases proportionally with age. After reaching sexual maturity, the priorities shift from adaptation and cognitive development to finding a mate, procreating, and caring for offspring, leading to a reduction in synaptic density and plasticity in the cerebral cortex (**Huttenlocher and Dabholkar, 1997**). The duration of reproduction and neurodegeneration in mammals is proportional regardless of differences in lifespan, suggesting that the aging process is similar across mammalian species, differing only in pace. This is likely due to a unique epigenetic clock that "ticks" at different rates, as evidenced by Horvath's algorithm, which determines a person's biological age with greater accuracy than chronological age based on a specific DNA methylation pattern at 353 selected CpG sites in the genome (**Horvath, 2013**). Unlike the epigenetic clock, which broadly reflects the general aging process, epigenetic drift is a unique collection of all acquired changes related to the environment in which an individual or cell culture ages. Some parts of this mechanism are common across all tissues, but it is known that each tissue can have its specific mechanisms (Hannum's epigenetic clock) and that during aging, the clock ticks faster for longer genes compared to shorter ones. Since pluripotent stem cells avoid age-related changes in DNA methylation, it is believed that reprogramming aged cells could lead to regeneration (epigenetic rejuvenation), but it is still unclear how to achieve rejuvenation without the risk of dedifferentiation.

The process of brain aging initiates at the cellular level, with distinct types of neurons aging at varying rates due to differential susceptibility to cellular aging mechanisms. Key alterations in gene regulation involve those encoding proteins responsible for synaptic function, responses to oxidative stress, and neuroinflammation (a state of low-grade inflammation due to heightened expression and activation of inflammasomes **[Figure 1]** and the secretion of pro-inflammatory cytokines). Alongside epigenetic modifications, the most significant changes in neuronal gene regulation include genomic instability (characterized by the accumulation of DNA damage and reduced efficiency of repair mechanisms, which are hallmarks of aged neurons leading to somatic mutations), alterations in mtDNA (mutations occur 10-20 times more frequently than in nuclear DNA due to the generation of reactive oxygen species during oxidative phosphorylation), and changes in the expression of genes associated with proteostasis, particularly those regulating autophagy and the ubiquitin-proteasome system for tagging and degrading damaged and misfolded proteins. The endothelial cells of brain capillaries maintain the integrity of the blood-brain barrier, which protects the brain from pathogens and other harmful factors. During aging, these cells are among the first to undergo transcriptional changes, likely due to

circulating signals. Indeed, heterochronic parabiosis experiments demonstrate that the plasma of aged mice accelerates the aging of brain capillary endothelial cells, while the plasma of young mice exerts a rejuvenating effect (**Chen et al., 2020**). The most significant rejuvenating interventions currently under investigation include metabolic interventions (metformin, mTOR antagonists, GLP-1R agonists), the removal of senescent cells, and epigenetic rejuvenation using Yamanaka transcription factors. A comparative analysis of gene expression in the brains of 19,300 individuals revealed that the primary "drivers" of brain aging are neuronal insulin resistance in the 40s (notably increased expression of the insulin-dependent glucose transporter gene *GLUT4* and decreased expression of the monocarboxylate transporter gene *MCT2*), vascular changes (reduced expression of *VEGFR1*), and heightened activation of innate immunity signaling pathways (increased expression of *APOE* and *IL1B* genes) (**Antal et al., 2025**). Therefore, future interventions targeting genes that are over or underexpressed during aging could represent a promising strategy for achieving longevity. A metabolic intervention study involving 101 participants demonstrated that ketones have a potent effect on re-stabilizing cortical network activity, with the maximum effect occurring between the ages of 40-60, suggesting that in midlife, carbohydrate intake should be reduced, while protein and healthy fat consumption should be increased, as has been indicated by the results of caloric restriction experiments for years.

Keywords: aging; brain; epigenetic drift; epigenetic clock; regulation of gene expression.

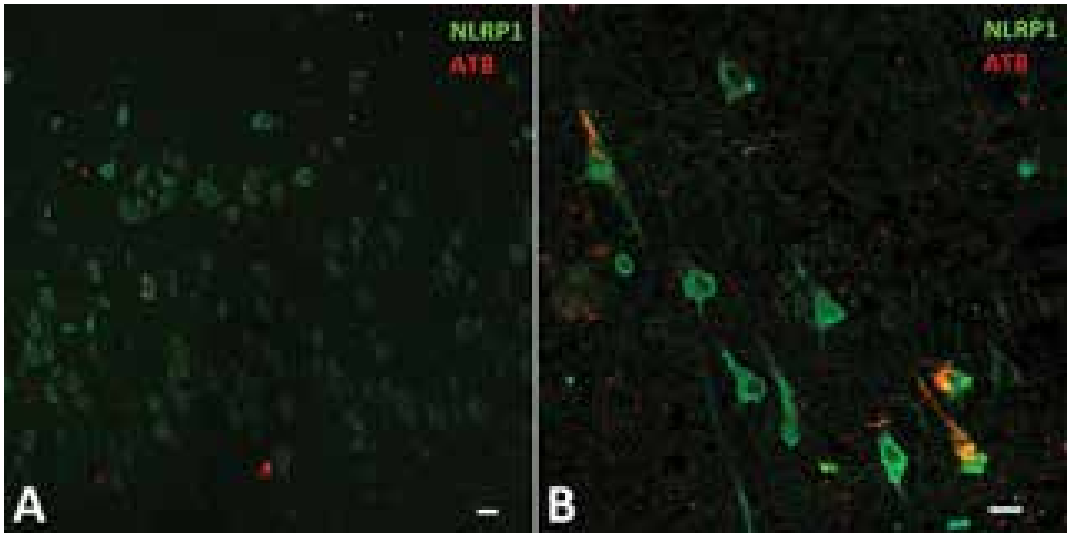

Figure 1. Expression of neuronal (NLRP1) inflammasome in the CA1 field of the hippocampus of a cognitively healthy 77-year-old woman (A) and a person with clinically and neuropathologically confirmed Alzheimer's disease aged 84 (B), whose disease lasted 3.5 years from diagnosis to death. Phosphorylated tau protein is visualized using AT8 antibody (binds to phosphorylated epitopes Ser199, Ser202, and Thr205). Scale bars = 20 μm.

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New possible treatments for dementia

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Abstract

Dementias are complex neurodegenerative and cerebrovascular disorders that necessitate distinct pharmacological strategies due to their diverse pathological mechanisms. Among the most prevalent forms are Alzheimer's disease, dementia with Lewy bodies, frontotemporal dementia, and vascular dementia. While no curative treatment currently exists to halt disease progression, recent advancements in Alzheimer's research have introduced therapeutic approaches aimed at slowing neurodegeneration.

A dominant framework in Alzheimer's disease pathophysiology is the amyloid cascade hypothesis, which posits that the accumulation of β-amyloid (Aβ) in the brain initiates a sequence of neurodegenerative events, ultimately leading to widespread neuronal degeneration and cognitive impairment. Aβ is a ubiquitous protein with several essential functions, including synaptic regulation, injury recovery, microangiogenesis, cell growth inhibition, antimicrobial activity, and maintenance of the blood-brain barrier. In individuals with Alzheimer's disease, Aβ accumulation begins 15 to 20 years before the onset of clinical symptoms. The etiology of this accumulation differs between early-onset and late-onset Alzheimer's; in early-onset cases, overproduction of Aβ drives its pathological aggregation, whereas in late-onset cases, impaired clearance mechanisms are primarily responsible. Current symptomatic therapies do not facilitate Aβ clearance; however, experimental and clinical studies involving monoclonal antibodies have demonstrated promising efficacy in reducing amyloid burden and slowing disease progression.

Recent FDA-approved monoclonal antibodies, such as lecanemab and donanemab, have garnered significant attention for their ability to target Aβ fibers, remove Aβ plaques, and mitigate cognitive decline. Clinical trials indicate that these therapies can slow cognitive decline by approximately 30 percent over 18 months. Lecanemab preferentially binds to Aβ protofibrils, preventing the formation of mature plaques, whereas donanemab binds to existing amyloid plaques, facilitating their rapid clearance via immune-mediated mechanisms. Both therapies, however, are associated with amyloid-related imaging abnormalities (ARIA), including cerebral oedema and microhemorrhages. Notably, the incidence of ARIA is lower with lecanemab compared to donanemab.

To address the limitations of first-generation monoclonal antibodies, trontinemab, a novel therapeutic currently in late-stage development, employs a brain shuttle mechanism that enhances blood-brain barrier penetration. Unlike lecanemab and donanemab, trontinemab incorporates a transferrin receptor binding domain, allowing superior brain penetration at lower doses, thereby improving efficacy while reducing systemic exposure and side effects.

Despite the therapeutic promise of monoclonal antibodies, their clinical application necessitates stringent patient selection. These treatments are indicated exclusively for early-stage Alzheimer's disease, specifically in individuals with mild cognitive impairment or mild dementia, representing only 10 to 20 percent of patients. They do not provide significant benefit in moderate or severe Alzheimer's disease, where extensive neurodegeneration has already occurred.

Prior to initiating treatment, confirmation of Aβ pathology via PET imaging or cerebrospinal fluid biomarkers is mandatory. Additionally, APOE genotype testing is strongly recommended due to its

significant influence on treatment safety. Carriers of the APOE ε4 allele, particularly homozygous ε4/ε4 individuals, exhibit the highest ARIA risk, with approximately 30 to 40 percent developing complications. Heterozygous ε3/ε4 carriers face a moderate risk, while non-carriers (ε3/ε3 or ε2/ε3) are at lower risk. Given these risks, APOE ε4 carriers require enhanced MRI surveillance throughout the course of treatment.

The efficacy and safety of monoclonal antibody therapy are also influenced by patient comorbidities. Individuals with a history of stroke, cerebral microbleeds, or severe cardiovascular disease are at an increased risk of ARIA and may not be suitable candidates. Furthermore, patients on anticoagulant therapy, such as warfarin or direct oral anticoagulants, face an elevated risk of intracranial hemorrhage. Those with severe hepatic or renal impairment may also have altered drug metabolism and clearance, necessitating careful assessment prior to treatment initiation.

Beyond clinical considerations, logistical challenges further limit widespread implementation. The administration of monoclonal antibodies requires specialized infusion centers, highly trained medical personnel, and frequent MRI monitoring, significantly increasing healthcare burdens. MRI scans are required at baseline and at regular intervals, such as at one, three, and six months' post-initiation, to monitor ARIA. Additionally, the high cost of treatment remains a major barrier, restricting access to only a subset of eligible patients.

In conclusion, monoclonal antibody therapy represents a significant milestone in the treatment of Alzheimer's disease, offering moderate yet meaningful cognitive benefits in select patient populations. However, stringent patient selection criteria, the risk of ARIA, the necessity for continuous MRI surveillance, and cost constraints pose substantial challenges to widespread clinical adoption. While these therapies mark a critical advancement in Alzheimer's disease management, their long-term clinical impact and cost-effectiveness remain areas of ongoing investigation.

Keywords: Alzheimer, amyloid, antibodies, lecanemab, donanemab

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News in the prevention and treatment of stroke

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Abstract

The leading cause of disability and mortality in Croatia and the world is stroke. Up to 90% of strokes can be prevented if ten modifiable risk factors are controlled. Stroke treatment has made significant progress in the last twenty years, primarily with mechanical thrombectomy, systemic thrombolysis, and other supportive measures in stroke units. Treatment in specialized stroke units increases the probability of a good outcome by 14%, the use of systemic thrombolysis by 30%, and mechanical thrombectomy by more than 50%.

This lecture aims to present news in research on stroke prevention and treatment, including the following research funded by the European Union: Validate, Umbrella, TRUSTroke, RES-Q+, POC4Triage, and EAST Stroke. These projects use artificial intelligence and/or advanced technology to improve stroke care from different angles.

Validate project uses artificial intelligence to revolutionize stroke treatment by analyzing patient data and helping physicians make faster, more informed decisions. To make artificial intelligence a reliable medical tool, VALIDATE focuses on 1) training artificial intelligence with diverse data to reduce bias, 2) collaborating with physicians, artificial intelligence experts, and patients, 3) following EU safety and ethical regulations, and 4) continuously testing for accuracy and reliability. The UMBRELLA project will use advanced technology, artificial intelligence, and real patient data to help physicians make better treatment decisions, improve recovery, and prevent future strokes. The UMBRELLA aims to improve stroke care by 1) faster diagnosis & treatment, 2) personalized care, 3) better access to care, and 4) understanding unknown strokes. The RES-Q+ project aims to improve the quality of stroke care by combining artificial intelligence with stroke data. RES-Q+ project will use technology to 1) automatically collect hospital data, 2) AI-powered virtual assistant tools will help physicians assess risks and improve treatment plans, 3) support recovery by tracking progress, and 4) provide information and ensure access to post-stroke care and rehabilitation. TRUSTroke project uses artificial intelligence to improve stroke recovery by helping predict future health risks. TRUSTroke project aims to develop an AI-driven system (easy-to-use application) to personalize treatment and reduce risks such as serious post-stroke health issues, mobility challenges, and recurring strokes.

The EAST-STROKE trial ('Early treatment of Atrial fibrillation for Stroke prevention Trial in acute STROKE') tests a new treatment strategy (rhythm control) for patients with acute ischemic stroke and atrial fibrillation. The EAST-STROKE trial aims to change that by integrating this treatment early after a stroke alongside standard care. The POC4Triage project will use advanced technology and artificial intelligence to improve the speed and accuracy of diagnosis in stroke and other cardiorespiratory conditions. POC4Triage is developing four innovative, portable medical devices designed to provide information for ambulance professionals and physicians to make faster treatment decisions in patients with acute stroke. These portable medical devices are 1) multi-diagnostic monitoring patch, 2) electroencephalography patch, 3) functional near-infrared spectroscopy (fNIRS) device for stroke monitoring, and 4) handheld blood test device (for biomarkers). The four devices will connect to a device hospital connectivity platform.

Keywords: stroke, stroke prevention, stroke treatment, trials

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Early habilitation in cerebral palsy and the role of robotic-assisted therapy

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Abstract:

Cerebral palsy (CP) is the most common motor disorder in childhood. In the last decade, major breakthroughs have been made in the early diagnosis, prevention, and intervention of CP, changing its incidence and prognosis, as well as the response to intervention. Severe motor impairments leading to immobility are becoming less common, meaning that more children than ever before can walk. The reduction in the incidence and severity of the clinical picture of CP is likely a result of a combination of extensive interventions during childbirth and neonatal intensive care, as well as early intervention programs during the period of greatest brain plasticity.

One of the key components of early habilitation is effective early diagnosis, which facilitates timely access to therapeutic interventions. Identifying children who may be at risk for CP involves standardized assessments and clinical criteria that effectively categorize their risks (McNamara et al., 2021). Delays in diagnosis often lead to missed opportunities for intervention, contributing to poorer long-term functional outcomes. In recent years, the body of evidence on early intervention in CP has continued to expand rapidly, providing professionals and families with the possibility of newer, safer, and more effective interventions. For instance, interventions initiated in infancy have been shown to leverage the peak neuroplasticity period, enabling children to achieve better motor and cognitive function (Li et al., 2023; Kwong et al., 2018).

Research supports the effectiveness of therapies that focus on promoting proper movement patterns and improving muscle control, such as robotic-assisted therapy. Robotic-assisted therapy has emerged as a promising intervention for children with CP, focusing primarily on enhancing gait and upper limb function. These therapies leverage advancements in robotics to provide structured and measurable rehabilitation that can be precisely tailored to individual patient needs. Particularly, robotic-assisted gait training has demonstrated beneficial effects by allowing children with CP to engage in repetitive movement patterns that improve gross motor function. Unlike traditional therapies, robotic therapy allows for training in much higher doses (performing a larger number of required movements) and higher intensity (number of movements per unit of time). This dosage per unit of time is considered a key factor in habilitation. This method can also provide objective measures for assessing treatment outcomes. A meta-analysis has confirmed that robotic gait training leads to significant improvements in mobility, indicating the effectiveness of these interventions in promoting locomotor skills (Conner et al., 2022). Further evidence suggests that robotic systems can help overcome specific deficits associated with CP, such as balance and endurance challenges, by enabling task-specific practice in a controlled and motivating environment. Additionally, interventions such as Constraint-Induced Movement Therapy (CIMT) are increasingly integrated with robotic systems. CIMT encourages intensive use of the affected limb by constraining the unaffected one, fostering functional improvements. With robotic systems adopting CIMT principles, children's engagement in task-specific training can enhance their manual abilities in daily life (Faccioli et al., 2023).

In conclusion, robotic-assisted therapy presents a valuable addition to the treatment landscape for children with CP, offering prospects for enhanced mobility, engagement, and measurable outcomes. Continued integration of these technologies into habilitation practices could shape the future of pediatric therapy for individuals affected by CP.

Keywords: early intervention, cerebral palsy, robotic-assisted therapy

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Deep Brain Stimulation – Clinical Experience, Indications, and Future Perspectives

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Abstract:

Deep brain stimulation (DBS) represents one of the most significant advancements in functional neurosurgery. Since its stereotactic origins in the mid-20th century, DBS has evolved into a standard neurosurgical procedure for various movement disorders, most notably Parkinson's disease, dystonia, and essential tremor. With over 210,000 devices implanted globally and more than 12,000 new procedures annually, the scope of DBS continues to expand.

At University Hospital Dubrava, more than 400 DBS procedures have been successfully performed to date. Depending on the indication, surgical targets include the subthalamic nucleus (STN), internal globus pallidus (GPi), ventral intermediate nucleus (VIM), posterior subthalamic area/caudal zona incerta (PSA/cZI), and the centromedian-parafascicular complex (CM-pf), as well as various thalamic nuclei in the treatment of chronic pain. In addition to movement disorders, DBS has demonstrated efficacy in treating Gilles de la Tourette syndrome and is emerging as a promising option in the management of disorders of consciousness (DOC).

Our multidisciplinary team has recently focused on the application of DBS in patients with DOC, including those in vegetative and minimally conscious states. Preliminary findings indicate that structural preservation of the thalamus, basal ganglia, and brainstem, along with a favorable gray matter proportion, may serve as predictive markers of clinical responsiveness to CM-pf stimulation. Although early results are encouraging, this indication remains under active investigation, requiring further studies to determine optimal patient selection and therapeutic mechanisms.

Despite its generally favorable safety profile, DBS is not without risks. Potential complications include intracerebral hemorrhage, infection, hardware malfunction, and neuropsychiatric side effects. Therefore, careful patient selection, thorough preoperative imaging, and multidisciplinary evaluation are essential to achieving optimal outcomes.

This abstract provides an overview of the historical evolution, current indications, and institutional experience with DBS, with particular emphasis on its expanding role in neuromodulation for patients with disorders of consciousness. The future of DBS lies in broadening its clinical applications, refining targeting strategies, and identifying reliable predictors of treatment response.

Keywords: Deep brain stimulation, movement disorders, disorders of consciousness, stereotactic neurosurgery, subthalamic nucleus

Neurotechnology in Psychiatry

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Neurotechnology is a rapidly evolving field at the intersection of neuroscience, engineering, and medicine, offering interconnected approaches to understanding and treating psychiatric disorders. Traditionally, key categories include neuroimaging, neuromodulation, and brain-computer interfaces (BCIs). Together, these technologies offer complementary insights: neuroimaging to observe brain patterns and activation of brain patterns in psychiatric disorders, neuromodulation to intervene in brain activity, and BCIs to interface with neural signals either in a one-way, or bi-directionally.
MRI-based techniques like fMRI, DTI and MR spectroscopy, or others like fNIRS, are employed to non-invasively visualize brain structure or function to identify neural correlates of mental disorders. Neuromodulation involves technologies that directly alter brain activity – for example, transcranial magnetic stimulation (TMS), vagal stimulation, deep brain stimulation (DBS), tDCS and tACS (transcranial direct/alternate current stimulation). These techniques use magnetic or electrical stimuli to influence brain areas or circuits linked to mood, cognition, or behavior. BCIs provide a direct communication link between the brain and external devices and were initially developed for neurological conditions (e.g. paralysis) but are increasingly relevant to cognitive enhancement and other psychiatric applications.
Clinical Applications
Neurotechnology is transforming both the understanding and treatment of psychiatric disorders. In depression, repetitive TMS applied to the dorsolateral prefrontal cortex is an established, FDA-approved treatment for patients who do not respond to medications. It is considered safe and effective, with about 50–70% of treatment-resistant cases achieving significant symptom relief, and considerably higher remission rates are reported when MRI-guided neuronavigation is used to personalize stimulation targets. Brain stimulation, an invasive neuromodulation mainly used in movement disorders, has been experimented in severe depression and obsessive-compulsive disorder. Notably, a recent closed-loop DBS approach – in which an implanted device delivers stimulation only when a neural signature of depression is detected – produced rapid and sustained remission in a patient with depression. This individualized, on-demand neuromodulation highlights the promise of precision neurotechnologies for psychiatry and the closer integration between the neuroradiology, the psychiatry and the neurosurgery. MRI-based neuroimaging genomics has revealed subtle but widespread brain alterations, as in schizophrenia and autism, reflecting the disorders' complex polygenic risk architecture. Such insights may guide biomarker development and early diagnosis in the future. In PTSD, neuroimaging consistently finds hyperactivity in fear circuits (e.g. amygdala) and hypoactivity in prefrontal regulatory regions, informing neuromodulation trials targeting those networks. Early studies with TMS in PTSD and anxiety suggest potential benefits, though results are mixed and further trials are ongoing.
Emerging therapies include neurofeedback and neurobiofeedback, a BCI-related technique where patients learn to modulate their own brain activity (via real-time EEG, preferably fNIRS or, theoretically, fMRI feedback), is being explored to alleviate PTSD, ADHD, and anxiety, capitalizing on neuroplasticity. Meanwhile, other BCIs are mostly in research phases for neurological impairment, but their rapid progress indicates their potential use in psychiatric use-cases. A recent breakthrough BCI enabled a person with paralysis to generate fluent speech by decoding cortical activity into words, demonstrating that high-bandwidth decoding of complex mental content is feasible. In principle, similar

interfaces might one day assist patients with psychiatric conditions. Evidence Base and Limitations: Some neurotechnologies are already backed by robust clinical trials (e.g. TMS for depression), whereas others remain experimental. Neuromodulation trials in disorders like schizophrenia and PTSD have had variable outcomes, underscoring that what works for one condition (or individual) may not readily translate to others. Also, many neuroimaging findings in psychiatry have not yet yielded actionable clinical tools, partly due to small effect sizes and heterogeneity. It is increasingly recognized that psychiatric illnesses involve distributed brain network dysfunctions rather than single lesions, which means interventions may need to be personalized and circuit-specific.
The trajectory of neurotechnology in psychiatry is clearly toward more personalized, objective, and biologically grounded care, complementing traditional pharmacotherapy with novel brain-based interventions.

Keywords: neurotechnology, neuroimaging, neuromodulation

How to Increase Treatment Effectivenes and Efficiency in Psychiatry?

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Abstract:
As approximately one third of psychiatric patients with serious mental disorders are treatment resistant and the other third shows only partial therapeutic response the question how to increase treatment effectiveness and efficiency is crucial one. Current psychiatry in our turbulent VUCA (*volatile*, *uncertain*, *complex*, *ambigous*) world is overwhelmed with the most varied disciplines, ideas, values, treatment techniques and methods, but short with comprehensive scientific theories and unfortunately not efficient enough. Different fields in psychiatry are oriented to different perspectives of mental disorders and their treatment: the disease/illness perspective, the perspective of the person, the cognitive perspective, the behavioral perspective, the narrative perspective, the spiritual perspective and systems perspective. Psychiatry should move from a pluralistic coexistence of the many separated brainless and mindless, not rarely even confronting disciplines to a coherent transdisciplinary and comprehensive mental health science and practice. Our genome operates within the context of the cell, the cell within the context of the body, the body within the context of the self, the self within the context of the society, and the society within the context of the universe. Human brain is where biological, psychological, social and spiritual processes and mechanisms meet and interact so psychiatric treatment should stimulate patients' brain cybernetics or psychocybernetics to work friendly and creatively for them. Transdisciplinary integrative psychiatry is the theory and practice of mental health care, research, treatment and prevention of mental disorders that 1.promotes the emphatizing therapeutic relationship between psychiatrists and patients and their families using shared decision making and a person-centered approach; 2.focuses on the whole person and total health, considering body/brain-mind-spirit and its systems inter-related with biological, psychological, social, cultural, ecological and spiritual aspects; 3.involves evidence-based practice and practice-based evidence and uses combination of different appropriate therapeutic methods and mental health disciplines; 4.eliminating illness and stopping pathogenesis as well as promoting salutogenesis and increasing wellness. Transdisciplinary integrative psychiatry with a „person life-story centered integrative diagnosis" approach is promising in search of appropriate answers to very relevant inter-individual variability in order to 1.close the gap between evidence-based medicine, value-based medicine, and narrative-based medicine with regards to effective care and valid clinical trials; 2.improve the course of mental disorders with earlier diagnosis and prevention measures; 3.improve adequate monitoring of vulnerability, resilience and psychological growth factors. As each patient is a unique, responsive and responsible subject creative, person-centered narrative psychopharmacotherapy with emphatizing cognitive-emotional-behavioral interaction with the patient evoking and expanding the patient's empathy, well-being and creativity may significantly increase treatment effectiveness. Arteficial intelligence offers many benefits to clinical psychiatry, treatment effectiveness and efficiency and professional and scientific development in psychiatry.

Keywords: treatment effectiveness, treatment resistance, transdisciplinary integrative psychiatry, emphatizing cognitive-emotional-behavioral psychotherapy

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Development of the Institute for the History and Philosophy of Science during 65 years since its foundation in 1960

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Summary:

Introduction: The paper presents the history and development of the Institute for History and Philosophy of Science of the Croatian Academy of Sciences and Arts on the occasion of 65 years of its existence. **Material and Methodes:** The startigpoint of this work were the Academy's yearbooks from the 1950s onwards, especially in tracking the sequence of employees in the title from assistant to academician of the Institute, which we organized in a table. **Results:** The Institute for the History of Natural, Mathematical and Medical Sciences, a new multidisciplinary institution, was established in 1960 consisting of four different foundations: the Department for the History of Medicine of the Academy's Institute for Medical Research, the Ruđer Bošković Memorial Museum in Lokrum, the Institute for the History of Medicine of the Faculty of Medicine in Zagreb, the Institute for the History of Pharmacy with its collection, and the Museum for the History of Healthcare of the Croatian Medical Association. The founder and first director of the Institute was Mirko Dražen Grmek, who was also the initiator of the publishing series Debates and material for the history of science, lounched in 1963. From the very beginning, the Institute established the methodology of work in the field of history of science in Croatia, its content and the profile of researchers. Its organization fell under the leadership of three Academy's departments: Department for Mathematical, Physical and Technical Sciences, later on Department for Mathematical, Physical and Chemical Sciences (heads: Željko Marković, Mihovil Proštenik and Žarko Dadić), the Department of Natural Sciences (head: Slavko Krvavica) and the Department of Medical Sciences (heads: Branko Kesić, Sergej Forenbacher and Vjekoslav Jerolimov). In 1992, on the initiative of academician Ivan Supek, the Department of Philosophy of Science joined the Institute, and the institution was renamed the Institute for History and Philosophy of Science. The Divisions of the Institute initially had individual directors (Žarko Dadić and Snježana Paušek-Baždar - Division for History of Natural and Mathematical Sciences; Biserka Belicza and Stella Fatović-Ferenčić – Division of Medical Sciences; Jasmina Lelas - Division for Philosophy of Science), and since 2020 Stella Fatović-Ferenčić has been appointed director of the entire Institute. The Institute was the driving force behind the establishment of the Croatian Museum of Medicine and Pharmacy HAZU, founded in 2014 by the Ministery of Culture.

Conclusions: Although with reduced personnel potential, the Institute for History and Philosophy of Science continues to monitor the development of scientific theories and their influence on the creation of knowledge in the fields of mathematics, physics, chemistry, biology, biomedicine and philosophy of science. It is a unique institution in Croatia that, by researching, presenting and preserving the

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This article was submitted to RAD CASA - Medical Sciences as the review article

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 27 April 2025
Accepted: 29 May 2025
Published: 25 June 2025

Citation:
Jerolimov V, Fatović-Ferenčić S. Development of the Institute for the History and Philosophy of Science during 65 years since its foundation in 1960

567=70-71 (2025): 144-153
DOI: 10.21857/yq32oh82q9

national scientific heritage, values the achievements of national development, harmonizing its activities with the fundamental mission of the Croatian Academy of Sciences and Arts.

Keywords: Croatian Academy of Sciences and Arts, Institute for History and Philosophy of Science – history, Mirko Dražen Grmek, history and philosophy of science - institutionalization of the history of science, Croatia

Sažetak:

Razvoj Zavoda za povijest i filozofiju znanosti tijekom 65 godina od njegova osnutka 1960.
Uvod: U radu se iznosi povijest i razvoj *Zavoda za povijest i filozofiju znanosti* Hrvatske akademije znanosti i umjetnosti u povodu 65 godina njegova postojanja. Prikazana je uloga Andrije Štampara i Mirka Dražena Grmeka u razvoju preteče ove ustanove *Odjela za historiju medicine* osnovane 1952. kao sastavnice Akademijinog Instituta za medicinska istraživanja. **Materijal i metode**: Okosnicu ovog rada činili su Akademijini ljetopisi od 1950-ih godina nadalje osobito u praćenju slijeda zaposlenika u zvanju od asistenta do akademika Zavoda koje smo prikazali i tablično. **Rezultati**: *Institut za povijest prirodnih, matematičkih i medicinskih znanosti* osnovan je kao nova interdisciplinarna ustanova 1960., a činili su ga : Odjel za historiju medicine Akademijinog Instituta za medicinska istraživanja, Spomen-muzej Ruđera Boškovića na Lokrumu, Zavod za povijest medicine Medicinskog fakulteta u Zagrebu, Institut za povijest farmacije sa zbirkom te Muzej za povijest zdravstva Zbora liječnika Hrvatske. Osnivač i prvi direktor Instituta bio je Mirko Dražen Grmek, ujedno i pokretač izdavačkoga niza *Rasprave i građa za povijest znanosti* koje počinju izlaziti 1963. godine. Od samih početaka Institut utemeljuje i definira metodologiju rada na području povijesti znanosti u Hrvatskoj, njezin sadržaj i profil istraživača te svojom organizacijom potpada pod vodstvo Akademijinog Razreda za matematičke, fizičke i tehničke znanosti, kasnijeg Razreda za matematičke, fizičke i kemijske znanosti (voditelji: Željko Marković, Mihovil Proštenik i Žarko Dadić), Razreda za prirodne znanosti (voditelj: Slavko Krvavica) i Razreda za medicinske znanosti (voditelji: Branko Kesić, Sergej Forenbacher i Vjekoslav Jerolimov).
Godine 1992. Zavodu se na inicijativu akademika Ivana Supeka priključuje Odsjek za filozofiju znanosti, te je ustanova preimenovana u *Zavod za povijest i filozofiju znanosti*. Odsjeci Zavoda isprva imaju pojedinačne upravitelje (Žarko Dadić i Snježana Paušek Baždar – Odsjek za povijest prirodnih i matematičkih znanosti; Biserka Belicza i Stella Fatović-Ferenčić - Odsjek za medicinske znanosti; Jasmina Lelas - Odsjek za filozofiju znanosti), a od 2020. upraviteljicom cjelovitog Zavoda imenovana je Stella Fatović-Ferenčić.
Zavod je pokretač osnutka *Hrvatskog muzeja medicine i farmcije HAZU* kojemu je inicijalna začetnica Stella Fatović-Ferenčić. Muzej je osnovan 2014., kao sedmi Akademijin muzej i prvi muzej znanosti u toj ustanovi te i nadalje usko surađuje sa Zavodom.
Zaključak: Premda smanjenim kadrovskim potencijalom, Zavod za povijest i filozofiju znanosti nastavlja pratiti razvoj znanstvenih teorija i njihov utjecaj na stvaranje znanja u području matematike, fizike, kemije, biologije, biomedicine i filozofije znanosti. Riječ je o jedinstvenoj ustanovi u Hrvatskoj koja istražujući, prezentirajući i čuvajući nacionalnu znanstvenu baštinu vrednuje dosege nacionalnog razvoja, usklađujući svoje djelovanje s temeljnom misijom Hrvatske akademije znanosti i umjetnosti.

Ključne riječi: Hrvatska akademija znanosti i umjetnosti, Zavod za povijest i filozofiju znanosti – povijest, Mirko Dražen Grmek, povijest i filozofija znanosti – institucionalizacija povijesti znanosti, Hrvatska

Introduction

This year we celebrate 65 years since the foundation of the Academy's Institute for History and Philosophy of Science. As the Institute was founded on the initiative of the historian of medicine Mirko Dražen Grmek in 1960, the commemoration took place as part of a symposium in the Library of the Croatian Academy of Sciences and Arts (HAZU), on January 9, 2025, precisely on Grmek's 101st birthday. On this occasion, we presented in this paper the history and development of this institution, unique in the territory of Croatia and in the wider environment, with an emphasis on its management and personnel structure until today. With regard to the prominent personalities who led it and worked in it, and the results that were created during the 65 years, we emphasized the need for its revitalization and role, with regard to nurturing scientific heritage and monitoring the epistemological, sociological, and humanistic aspects of the science development to this day.

The backbone of this work were the Academy's yearbooks from the 1950s onwards, especially in tracking the sequence of employees in the title from assistant to academician of the Institute, which we also presented in a Table 1. During the years, assistants - trainees, young researchers and scientific novices were employed at the Institute over the years: Nevenko Boroš, Ivanka Mustač, Marin Šubarić, Melita Rastija, Jadranka Boljunčić, Snježana Šain, Ana Borovečki, Tatjana Buklijaš, Vanja Flegar, Neva Grgičević-Mihalić and Vedran Duančić. The aforementioned were not included in the table, because they were not in a permanent casual relationship at the Institute.

Section for the History of Medicine of the Institute for Occupational Hygiene – Forerunner of the Institutionalization of the History of Science in the Yugoslav Academy of Science and Arts (YASA)

The 1950s brought reorganization in the then JAZU, so in 1946 and 1947 the Academy's departments were founded.[1] For example, the III Department of Natural and Medical Sciences of the JAZU was founded, and the first regular members of that department were the otolaryngologist Branimir Gušić and the dermatovenerologist Franjo Kogoj (1947).[2] However, not all physicians belonged to the III Department, so Andrija Štampar, a public health strategist and one of the founders of the World Health Organization, was elected as a regular member of I Department of Philosophy and Social Sciences in 1947.[3] After World War II, Štampar was the director of the School of Public Health in Zagreb, dean of the Faculty of Medicine of the University of Zagreb, rector of Zagreb University (1945–1946) and president of YASA (1947–1958). [4] As an envoy of the World Health Organization, he studied public health and medical education in Afghanistan, Ethiopia, Egypt, Sudan, and from his travels and experiences in the field, he could certainly see how important knowledge of the culture and history of each individual nation is in accessing health and disease prevention. It was a time when his public health models of health care, confirmed in the period between the two world wars, were taking off through the social and health care of the population in the entire territory of the former Yugoslavia.[4-6] Particular care was taken of to protect the health of the working population, which is confirmed by the fact that precisely on Štampar›s initiative, on December 27, 1947 by the decision of the Yugoslav Academy of Sciences and Arts, the Institute for Occupational Medicine was founded, which already had 34 employees at the beginning. During 1948, all preparations for the work of the Institute were carried out, and a year later, the scientific research activity of this institution officially began. [7]

At the same time, Mirko Dražen Grmek, a young doctor, completed his studies at the Faculty of Medicine of the University of Zagreb, who, thanks to his professor role models, Andrija Štampar and Lujo Thaller, gradually decided to research the history of medicine. He chose that field of medicine, which he saw as a kind of counterbalance to the processes of fragmentation in medicine, but also as an opportunity to connect biomedical, natural and humanistic sciences.[8] Štampar quickly recognized Grmek's ambitions and invited him to organize the Section of History of Medicine within the Institute for Occupational Hygiene at YASA, which Grmek readily accepted. The YASA Yearbook for 1952 notes that at the session of the Institute's Expert Council on July 4, 1952, it was concluded that it would consist of: Section of Biochemistry, Section of Leptospirosis, Section of Oncology, Section of Viruses and Rickettsia, Section of Medical Parasitology, Section of Zoonoses, and Section of History of Medicine.[9] In the description of the work of the last unit within the Institute, it is stated that the Section for the History of Medicine shall study materials from various State Archives, work on historical-medical articles for the Encyclopaedia of Yugoslavia as well on the editing of the Medical encyclopedia.[9] Thanks to the report that he submitted to the Academy after his field research in Zadar, which took place from April 1 to June 28, 1952, it is possible to realize the ambitious work plan that Grmek outlined at the beginning of his employment. It is visible that he devoted the most important part of his research to the study of documentation on health conditions in Dalmatia during the French rule (1806-1813) and data on the history of medical and pharmaceutical courses at schools in Zadar and Trogir. [10] Those topics were the main goal of his research and the starting point for the preparation of his future doctoral dissertation. The materials stored in the Zadar archive, library and archaeological material opened his eyes to numerous areas, which he would later cover in his works. The richness of the material created an insight into the scope of the yet unexplored medical heritage.

During 1953, the Academy attached all its units that dealt with biomedicine to the Institute for Occupational Medicine,

Table 1. Heads, directors and researchers of the Institute for the History and Philosophy of Science

HEAD	DIRECTOR	RESEARCHER	INSTITUTION / LOCATION
Željko Marković (1960 - 1974)	Mirko Dražen Grmek Institute Director (1960 -1963)	Hrvoje Tartalja (1960 - 1977) Lavoslav Glesinger (1960 - 1975) Žarko Dadić (1960 – 2023) Ilinka Senčar -Ćupović (1960 - 1994) Renata Peroš (1960 - 1977) Antun Dorn (1960 – 1963)	**Institute for the History of Natural, Mathematical and Medical Sciences** Zagreb, Opatička 18 Zagreb, Demetrova 18
	Hrvoje Tartalja Institute Director (1964 - 1977)	Vladimir Dugački (1966 - 1969) Biserka Belicza (1969 - 2005) Marija Weiss (1974 - 1977) Snježana Paušek- Baždar (1974 - 2019) Josip Balabanić (1975 - 1988) Zdenka Šušnić-Fliker (1978 - 1989.)	
Brako Kesić (1979 - 1982) **Mihovil Proštenik (1982 – 1986)** **Slavko Krvavica (1986 - 1990)** **Sergej Forenbacher (1990 - 1992)**	Biserka Belicza Division Director (1980 - 2005)	Biserka Belicza (1969 - 2005) Zdenka Šušnić-Fliker (1978 - 1989) Stella Fatović-Ferenčić (1985 - 2025) Željko Dugac (1997 -)	**Institute for the History of Natural, Mathematical and Medical Sciences – *Division for the History of Medical Sciences*** Zagreb, Demetrova 18
Žarko Dadić (1992 - 2023)	Žarko Dadić Division Director (1982 - 1995) Snježana Paušek-Baždar Division Director (1996 - 2019)	Snježana Paušek- Baždar (1974 - 2019) Josip Balabanić (1975 - 1988) Stipe Kutleša (1984 - 2000) Marijana Borić (1991 -) Branko Hanžek (2003 - 2023)	**Institute for the History and Philosophy of Science – *Division for the Natural and Mathematical Sciences*** Zagreb, Ante Kovačića 5
	Jasmina Lelas Division Director (1992 - 1998)	Zdravko Radman (1992 - 1994) Tihomir Vukelja (1992 - 1998) Bojan Marotti (1992 - 2022)	**Institute for the History and Philosophy of Science - *Division for the Philosophy of Science*** Zagreb, Ante Kovačića 5
	Biserka Belicza Division Director (1980 - 2005) Stella Fatović-Ferenčić Division Director (2005 - 2019) & Institute Director (2020 - 2025)	Stella Fatović-Ferenčić (1985 - 2025) Željko Dugac (1997 -) Martin Kuhar (2009 -)	**Institute for the History and Philosophy of Science – *Division for the History of Medical Sciences*** Zagreb, Demetrova 18 Zagreb, Gundulićeva 24
Vjekoslav Jerolimov (2024 -)	Stella Fatović-Ferenčić Institute Director (2020 - 2025)	Stella Fatović-Ferenčić (1985 - 2025) Željko Dugac (1997 -) Martin Kuhar (2009 -)	**Institute for the History and Philosophy of Science – *Division for the History of Medical Sciences*** Zagreb, Gundulićeva 24
		Marijana Borić (1991 -)	**Institute for the History and Philosophy of Science – *Division for the Natural and Mathematical Sciences*** Zagreb, Ante Kovačića 5

so the Institute changed its name to the Institute for Medical Research.[11] It had seven subunits, including the Section for History of Medicine, which was located at Demetrava Street 18 in Zagreb. The Director of the Department was Mirko Dražen Grmek, with whom one administrative person works - a typist. Establishing the methodology of scientific research within the field he deals with, Grmek continues his research in the State Archives and various libraries, collects data for the Croatian medical bibliography.[12] He published most of this material while he was working in Croatia. Most importamtly we cite his doctoral dissertation Medical Faculties in Dalmatia in the French Era reign (1806-1813), which he defended in 1958, becoming the first doctor of science in this field. It was an important step in the struggle to position the history of medicine as a scientific discipline at the University of Zagreb. In addition together with Štampar, Grmek compiled a script and then a manual Introduction to Medicine for students at the beginning of medical studies, which would later go through three editions (1961, 1971, 1996). he obtained an assistant professorship, and in 1960 he became a professor at the Faculty of Medicine of the University of Zagreb. [13]

In an era in which the fragmentation of medicine is intensifying in accordance with the development of specialist and subspecialist areas, Grmek develops views on a broader view of the incidence and approaches to the treatment of diseases, on the processes of forming scientific theories and ideas about science, and becomes a recognizable personality in the field of the history of science in the entire territory of Yugoslavia at the time. In this sense, the logical sequence of his activities was aimed at the foundation of an institution that would interdisciplinaryly deal with that topic in the field of connecting basic and biomedical sciences.

Foundation of the Institute for the History of Natural, Mathematical and Medical Sciences Jazu

At the end of the fifties of the last century, Grmek's intention to connect individual institutions that dealt with the history of science was realised on May 7, 1959 with the decision of the JAZU Presidency on the establishment of the Institute for the History of Natural, Mathematical and Medical Sciences. Then his tasks are defined: *to organize, encourage and carry out scientific work in the field of history of natural, mathematical and medical sciences, with special regard to the development of these sciences in South Slavic countries, primarily in Croatia; to develop and improve historical methodology in the field of sciences represented in the work of the Institute; to collect and process archival, bibliographic and museum materials related to the scientific activities of the Institute and to take care of the maintenance and development of such collections within the Institute, so that they are trained for scientific use; to work on the improvement of scientific and professional personnel and the creation of scientific and professional youth; to publish the results of his scientific and professional work in periodical and other publications of the Institute, taking into account their significance for the national, social and cultural interests of the country; to provide assistance in matters of organization and conduct of teaching in the history of sciences that fall within the scope of work of the Institute, especially to help the improvement of such teaching at the appropriate faculties of the University of Zagreb and other higher education institutions with its recommendations.* [14]

Fundamental preparations for the organization of the Institute were thus started, however, its organization was carried out only in 1960. The core of the Institute was the Section for the History of Medicine of the Institute for Medical Research, which with its staff and inventory was transferred to the newly founded Institute on January 1, 1960. On June 10, 1960, the leadership of the Institute was entrusted to Mirko Dražen Grmek as permanent director (Figure 1). In January 1960, the Ruđer Bošković Memorial Museum in Lokrum was added to the Institute, and on March 1, 1960, the Institute for the History of Medicine of the Faculty of Medicine in Zagreb was taken over. On May 17, 1960, based on an agreement with the Pharmaceutical Society of Croatia, it was incorporated into this Institute and the previous Institute for the History of Pharmacy with its collection. Finally, on December 13, 1960, the Croatian Medical Association handed over the material of the Museum for the History of Healthcare to the Institute for permanent use and safekeeping. During 1960, the Council of the Institute was formed, and the academician Željko Marković was elected as the president of the Council, who soon after assumed the role of the head of the Institute. Based on the announced competition, three senior scientific associates (Prof. Dr. Lavoslav Glesinger, Prof. Dr. Mirko Dražen Grmek and Assistant Professor Dr. Hrvoje Tartalja) and three assistants (Žarko Dadić, Antun Dorn and Renata Peroš) were selected. Independent administration and accounting operations of the Institute were also set up. [15]

The Institute was located in Zagreb's Upper Town, in two locations, at Demetrova Street 18 and Opatička Street 18. In Demetrova Street, the natural and medical sciences were located, and in Opatička Street, the pharmaceutical collection was located, led by Hrvoje Tartalja. From the very beginning, all professions, united in the Institute, work with great enthusiasm. It begins with arranging the museum material, books, photo library and bibliographic files. The Institute cooperates with various institutions, primarily with the Medical Faculties in Zagreb and Rijeka, the Lexicographic Institute, the Faculty of Pharmacy in Zagreb and institutes for the history of science abroad. Employees present the results of their research at national and international conferences, as well as in professional and scientific periodicals. On the initiative of Mirko Dražen Grmek, the Institute's publishing initial book series entitled *Debates and material for the history of science* (*Rasprave i građa za povijest znanosti*) were

launched in 1963, with the first book edited by Hrvoje Iveković and Mirko Dražen Grmek. From 1963 to 1969, the *Debates* were published in a single volume of the entire Institute, while from book 4 (1983) a special edition of the Academy's Department for Mathematical, Physical and Technical Sciences was published, and from book 5 (1989) a special edition of the Department for Medical Sciences was published. So far, 21 books have been published. The contents of these books, which will soon be fully digitized, have grown into reference literature and a fundamental starting point for research into the history of science in our region.[16]

Figure 1. Mirko Dražen Grmek (Krapina, 2024 – Paris, 2000), the founder of the Institute. Bust (40 x 27 x 25 cm; No. HMMF - 4344).

After Grmek's Departure from Croatia

During 1963, there were changes in the personnel composition of the Institute, since the three-year directorship of the then director M. D. Grmek expired on June 10, 1963. Given that he was called to settle the legacy of Claude Bernard, Grmek leaves Croatia and goes to live and work permanently in Paris. In the same year, assistant archaeologist and art historian Antun Dorn left the Institute. In 1964, Hrvoje Tartalja was appointed director of the Institute, and Nevenko Boroš, a graduate philosopher, was appointed assistant, while dr. Žarko Dadić was transferred from Dubrovnik to Zagreb upon request.[17] The new director of the Institute, Hrvoje Tartalja, brings a new research and museum dimension to the Institute. He started to deal with the history of pharmacy after the Second World War, he was also the founder of the Section for the History of Pharmacy, which became a member of many international organizations during his leadership. In 1950, with Tartalja's efforts, the compulsory subject History of Pharmacy was introduced into the curriculum of the Faculty of Pharmacy, and with the help of the newly founded Pharmaceutical Society of Croatia, the Institute for the History of Pharmacy was founded on April 19, 1952. The Institute organized the collection by taking over objects that were in the Pharmaceutical Society of Croatia and in other institutions, and was located at Tomašićeva Street 12 in Zagreb. However, Tartalja was not satisfied with the development of that institution, nor with the previous collection status therefore he joined the Institute for the History of Natural, Mathematical and Medical Sciences at Grmek's invitation, which he headed after Grmek's departure to Paris. [18] In addition to Tartalja, the Institute for the History of Natural, Mathematical and Medical Sciences received Renata Peroš, pharmacyst, who will later hold the position of head of the library and archives.[19] The psychiatrist Lavoslav Glesinger, and a historian of medicine, who after Thaller taught the history of medicine at the Faculty of Medicine in Zagreb from 1946, worked as Hrvoje Tartalja's deputy, and worked at the Institute from 1960 to 1975, when he retired. Mathematician Žarko Dadić and chemist Ilinka Senčar-Ćupović also worked at the Institute at that time. Since 1966, Vladimir Dugački has also worked at the Institute, who primarily works on bibliographies, and after his departure in 1969, Biserka Belicza, doctor of medicine, joined the Institute, who then began researching the history of medicine under the mentorship of Lavoslav Glesinger. Since December 1974, Snježana Paušek-Baždar has been employed at the Institute as an assistant and historian of chemistry. In March 1974, another pharmacyst, Marija Weiss, was admitted to the Institute, who was involved in research on the organization of the pharmacy service in Istria since the 13th century. At the Institute research related to the history of healthcare in Vojna Krajina, the history of healthcare in Istria, and research into the history of exact sciences in Croatia continues. [19]

The head of the Institute until the end of 1974 was Željko Marković from the Department of Mathematical Sciences. In 1975, Josip Balabanić was employed at the Institute for the History of Natural, Mathematical and Medical Sciences as an assistant for the history of biological sciences. This scientist of the Institute also created a doctoral dissertation, entitled Darwinism in Croatia until 1918, under the mentorship of M. D. Grmek, and defended it in 1980. Balabanić worked at this institution until 1988, when he was employed at the Natural History Museum in Zagreb. In the period from 1960 to 1974, as part of the

History of the Croatian Nation project, the Institute conducted research into the history of natural, mathematical and medical sciences in Croatia, which in 1977 was divided into two themes: "History of exact and natural sciences in Croatia" and "History of medicine and pharmacy in Croatia". A total of eight researchers worked on this task.

During 1977, there were more drastic changes in the Institute's research staff. Namely, assistant Marija Weiss mouved to another work organization, and Renata Peroš, senior professional associate, retired. At the same time, Hrvoje Tartalja, scientific advisor, retired too, so the filling of empty positions has been initiated. At the same time Biserka Belicza spends the academic year 1978/1979. in the postdoctoral study of history of science and history of medicine at Harvard University (USA). [19] In 1978, the Division for the History of Medical Sciences employed Zdenka Šušnić-Fliker, who deals with research into the scientific research aspects of Croatian physicians, the historiography of the development of professional societies and journals, and individual personalities in the field of pharmacy. She completed postgraduate studies in the history and philosophy of science in Dubrovnik, where Mirko Dražen Grmek was also teaching at the time. Zdenka Šušnić-Fliker's master's degree is mentored by Mirko Dražen Grmek, who, despite moving to France, still maintains contact with our scientists.[19]

Two Divisions, Two Directors, Two Location

In the eighties of the last century, it was proposed to the Presidency of the Academy that the Institute for the History of Natural, Mathematical and Medical Sciences of the Research Center of the Academy should be separated into two research units, namely: the Institute for the History of Natural and Mathematical Sciences under the jurisdiction of the Department for Mathematical, Physical and Technical Sciences, and the Institute for the History of Medicine and Pharmacy under the jurisdiction of the Department for Medical Sciences. However, the Presidency decided not to split the Institute into two research units, but to establish two divisions: the Division for the History of Medical Sciences and the Division for the History of Natural and Mathematical Sciences within the Institute. The first one is led by associate member Biserka Belicza, and the second department associate member Žarko Dadić. It was further decided that the Head of the Institute for a period of three years would be proposed by the involved Departments of the Croatian Academy of Sciences and Arts (II, III and IV), and that, according to the Presidency's conclusion at the time, the first head would be proposed by the Department of Medical Sciences. At the proposal of the Department of Medical Sciences, the Presidency of the Academy elected academician Branko Kesić as head of the Institute for the History of Natural, Mathematical and Medical Sciences of the JAZU Research Center. [20]

The divisions were also physically separated in such a way that the pharmaceutical collection and library from Opatička 18, which was led by Hrvoje Tartalja, was attached to the Medical Division located at Demetrova 18, and the Mathematics and Natural Sciences Division was moved to Ante Kovačića Street 5, where it is still located today. In the Division for the History of Natural and Mathematical Sciences, Žarko Dadić, scientific advisor, continues his research on the history of mathematics, physics and astronomy, Ilinka Senčar-Čupović, scientific associate and Snježana Paušek-Baždar, scientific assistant, are engaged in research on the history of chemistry, Josip Balabanić, scientific assistant, studies the history of biology and in 1980 he obtained his doctorate. During 1984, Stipe Kutleša joined the work of this Division, who graduated in history and philosophy at the Faculty of Philosophy in Zagreb in 1979, and in physics at the Faculty of Science and Mathematics in 1982. Jadranka Merlin is working on organizing the reference library and creating a catalog of works from the history of science. [21]

In the Division for History of Medical Sciences, Biserka Belicza, senior research associate, studies the history of medicine, Zdenka Šušnić-Fliker, assistant, studies the history of pharmacy. The Institute also employs Jadranka Tokić as an administrator and Jelica Trčak as an assistant clerk. The task and work of the Division for the History of Medical Sciences continued to aime at creating a foundation for the systematic study of the history of medicine and pharmacy, health conditions and the health culture of the people of Croatia. [21] In the mid-1980s, in the Division for History of Natural and Mathematical Sciences, work was carried out on the project "Development of Marxism and its current theoretical currents" with the program "Research of the history of mathematics, physics, astronomy, chemistry, biology, geology and technology in Croats", which was financed by SIZ-VII., "Croatia through the centuries — sources, aids and contributions to history", which was financed by SIZ VII and part of the program from the Basic Plan of the Academy.[21] In December 1985, Stella Fatović-Ferenčić, doctor of medicine, was hired in the Division for History of Medical Sciences. She previously wrote a thesis on the history of the plague in Croatia under the mentorship of Biserka Belicza, which she defended at the Faculty of Medicine of the University of Zagreb in 1984. In October 1989, Jadranka Boljunčić came to the Institute for History of Natural, Mathematical and Medical Sciences as an assistant trainee for the history of biology. In the same year, Snježana Šain was employed as a temporary assistant trainee in the Division for History of Medical Sciences, while Zdenka Šušnić - Fliker terminated her employment in the Division on October 2, 1989. [22]

During the 1980s, several heads of the Institute were replaced. After academician Branko Kesić, the Institute was led by an extraordinary member of the Academy, later academician Mihovil Proštenik (1982-1986), an extraordinary member of the Academy, later academician Slavko Krvavica (1986-1990), and academician Sergej Forenbacher (1990-1992). [23]

Change of the Institute's Name and a New Research Unit Addition

At the session in 1991, the Presidency of the Academy supported the initiative of the Academy's departments, in which the majority of members declared that the name of the Academy should be changed from the then Yugoslav Academy to the Croatian Academy of Sciences and Arts. The very next year, in 1992, the Division for Philosophy of Science was added to the newly named Institute for History and Philosophy of Science, which came from the Institute of Philosophy of Science and Peace, which was then headed by academician Ivan Supek. That Institute was also the administrative center of the Yugoslav Pugwash Conference of which Supek was the president since its foundation. Employees of different professions worked in the Division of Philosophy of Science: Jasmina Lelas, who dealt with the theory of the development of science, and was also the director of the Division of Philosophy of Science, after it was merged with the Institute for History and Philosophy of Science (until 1998, when she died), then Zdravko Radman, who dealt with the philosophy of science and was also active in the Pagwash movement, then Tihomir Vukelja, who dealt with the philosophy of physics, and Bojan Marotti, who deals with the philosophy of language, the theory of meaning and the history of Croatian philosophy.[23, 24]

In 1992, academician Žarko Dadić became the head of the newly created Institute for History and Philosophy of Science, who intensively dealt with the history of exact sciences in Croats for a number of years, which by then resulted in several published syntheses: Marini Ghetaldi Opera omnia (1968), Marin Getaldić, Collected Works, I., (1972), History of Exact Sciences in Croats (1982), Ruđer Bošković (1987), Exact Sciences of the Croatian Middle Ages (1991), History of Ideas and Methods in Mathematics and Physics (1992), Croats and Exact Sciences at the Dawn of the Modern Age (1994), Exact Sciences in Croats in the Age of Enlightenment (1994).[25] Žarko Dadić's deputies in that period were Biserka Belicza, manager of the Division for History of Medical Sciences, and Jasmina Lelas, manager of the Division of Philosophy of Science. In addition to the aforementioned, Ilinka Senčar-Čupović, Zdravko Radman, Snježana Paušek-Baždar, Stipe Kutleša, Stella Fatović, Bojan Marotti, Marijana Buljan-Klaić, Tihomir Vukelja, and Snježana Šain also worked at the Institute.

Žarko Dadić retired in 1995, and in 1996 the chemist Snježana Paušek-Baždar took his place as director of the Division for the History of Natural and Mathematical Sciences. The Division continues to work on the project "History of Natural and Mathematical Sciences in Croats" (principal researcher Academician Žarko Dadić), which contains a program of research into the history of mathematics, physics, astronomy, chemistry, biology and technology in Croats, and is financed by the Ministry of Science and Technology of the Republic of Croatia. [26]

In that period, the work of the Division for the History of Medical Sciences was focused on the study of the development of medical scientific thought; research on the state and problems of medical practice and health care; analysis of the health status and health culture of the inhabitants of Croatia throughout the centuries. During 1998, Tatjana Buklijaš and Željko Dugac came as novices to the Study of Croatian Medical Heritage project, led by Biserka Belicza in the Division for History of Medical Sciences. [27] Tatjana Buklijaš will work here intermittently due to her postgraduate education abroad until mid-2005. In 1999, Stella Fatović-Ferenčić started an inter-academic project (Croatian Academy of Sciences and Arts and Austrian Academy of Sciences) named Iconography in Dermatovenerology, as part of which two international books and a number of papers were published. [27]

Institute for History and Philosophy of Science in the Period 2000 - 2025

The Institute welcomed the third millennium with reduced capacities. In addition to Snježana Paušek-Baždar, others work in the Division for History of Natural and Mathematical Sciences Marijana Buljan-Klaić (later M. Borić), and since 2003 Branko Hanžek. Snježana Paušek-Baždar works on the History of Alchemy in Croats project, which is financed by the Ministry of Science, Education and Sports of the Republic of Croatia. Marijana Buljan-Klaić participates in the project History of Natural and Mathematical Sciences in Croats (principal researcher Academician Žarko Dadić), which is financed by the same Ministry. Branko Hanžek works on the project Croatian medical identity and its European context (principal researcher Stella Fatović-Ferenčić). In the Division for History of Medical Sciences, of the new recruits who worked on the Biserka Belicza project, including Ana Borovečki who arrived in 2000, only Željko Dugac is employed for an indefinite period. The director of the Division, Biserka Belica, died in April 2005, so Stella Fatović-Ferenčić was appointed as the new director of the Division. The function of the Division continues with the relocation of the library, archive and museum inventory to new premises at Gundulićeva Street 24 in Zagreb. In 2005, the research student Neva Grgičević-Mihalić, was employed on the Iconography in Medicine project, which will remain in that capacity for the next three years. After her, Martin Kuhar joined the Croatian Medical Identity and its European Context project as a newcomer. [23]

After a break of 15 years, the Division for History of Medical Sciences, in cooperation with the Department of Medical Sciences of the Academy, revitalized the publication of the publication *Discourses and Materials for the History of Science* with the eighth book of the publishing series dedicated to Mirko Dražen Grmek. The editors of the new edition of the publishing series were Marko Pećina and Stella Fatović-Ferenčić, under whose editorship a total of eight books have been published so far. [16]

The revitalization of this edition within the Division for History of Natural and Mathematical Sciences is due to Snježana Paušek-Baždar, who, together with academician Ksenofont Ilakovac

from the Department of Mathematical, Physical and Chemical Sciences of the Academy, is revitalizing the publication of six books of this publishing series.[16]

In the Division for History and Philosophy of Science, several new novices participate in various projects. In the Division for History of Natural and Mathematical Sciences, Vanja Flegar continues her work in history of chemistry in 2013, and after returning from maternity leave, continues to work part-time. During 2014, the Division of Philosophy of Science includes Bojan Marotti and Željko Dugac, as well as postdoctoral student Vedran Duančić. The latter participate in the project Croatian Scientific and Philosophical Heritage: Transfers and Appropriations of Knowledge from the Middle Ages to the Twentieth Century in the European Context of the Croatian Science Foundation, led by Željko Dugac.[23] In 2015, Martin Kuhar defended his doctoral dissertation entitled Eugenics in Croatian medicine and its impact on the public in the period from 1859 to 1945, under the mentorship of Stella Fatović-Ferenčić, and in 2019 he was accepted to the Division for History of Medical Sciences.

At the very beginning of her directorial mandate, Stella Fatović-Ferenčić initiated the project of establishing the Croatian Museum of Medicine and Pharmacy HAZU, which was allocated the space of the former printing house at Gundulićeva street 24, and which was formally established by the decision of the Ministry of Culture of the Republic of Croatia in 2014. During 2015, the Museum's curator and its head Silvija Brkić Miđić, was employed in the premises of the Division for the History of Medical Sciences at Gundulićeva 24 (3rd floor). In terms of its mission, activity and content, the museum is closely related to the work of the Division for the History of Medical Sciences of the Institute, from which it was born, and develops and functions accordingly. Academician Marko Pećina was appointed head (2015) and Stella Fatović-Ferenčić, the director of the Division, deputy head of the Museum (2016). She is also the co-author of its museological concept (2016) and the script for the permanent exhibition (2018). [28] By the Decision of the Academy's Management in 2020, Stella Fatović-Ferenčić was appointed director of the entire Institute for History and Philosophy of Science, who is now leading it already in her second term.[29] During 2022, the Division for History of Medical Sciences was moved from Gundulićeva 24 to Veliko Trgovišće due to the reconstruction of the building as a result of the earthquake. The return is expected in 2025. Since two more employees (Bojan Marotti and Branko Hanžek) retired from the Institute in 2023, four employees remain in the Institute today, in three divisions: Stella Fatović-Ferenčić, Marijana Borić, Željko Dugac and Martin Kuhar.

In March 2024, academician Vjekoslav Jerolimov, the first representative of dentistry in the Department of Medical Sciences of the Academy, was appointed head of the Institute.[30] He deals with prosthodontics, temporomandibular disorders and dental materials, and more recently sports dentistry and the history of dentistry. Bearing in mind the significant reduction in the number of scientists at the Institute in recent years, the new head, in agreement with the Academy's Management, will try to change this situation, thereby contributing to the mission and scientific role of the Institute, and also of the Academy itself.

Conclusion

The work of the Institute for the History and Philosophy of Science began 65 years ago with the aim of exploring the history of science in Croatia. Over time, it changed names and organization (incorporating the Division of Philosophy of Science) as well as locations (Demetrova 18, Opatička 18, Ante Kovačića 5, Gundulićeva 24). It was under the leadership of the Academy's Departent for Mathematical, Physical and Technical Sciences, later Department for Mathematical Physical and Chemical Sciences (academicians Marković, Proštenik and Dadić), Department for Natural Sciences (academician Krvavica) and Department for Medical Sciences (academicians Kesić, Forenbacher, Jerolimov). Employees of the Division for History and Philosophy of Science continue to monitor the development and structure of scientific theories and their impact on the creation of knowledge in the fields of mathematics, physics, chemistry, biology and biomedicine, comprising the results of their research in a global context. All three components of the Institute complement each other with their research, establishing links between the history of science and the history of philosophy. By evaluating national achievements in the history of science and monitoring the impact of the development of science and technology on social justice, politics and democracy, the results of their research continue to question the ethical implications and challenges in science and advance the understanding of science and its development model. The institute is a unique such institution in Croatia, which by researching, presenting and preserving the national scientific heritage values the achievements of national development, harmonizing its activities with the fundamental mission of the Croatian Academy of Sciences and Arts.

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Book review: Marko Pecina et al. SPORTS MEDICINE, 2nd Edition

Author:
Mihovil Plečko

In January 2025, the 2nd edition of the book *Sports Medicine*, authored and edited by Marko Pećina and his collaborators, was published. This new edition is rightly called the *2nd supplemented and revised edition* because, in addition to the 39 chapters from the first edition, seven new chapters have been added. Notably, these include a chapter on preserving mental health in professional athletes and a chapter on para-athletes. All chapters from the previous edition have undergone either significant or minor revisions and updates to the text, images, and tables, with every chapter enriched by recent literature references.

The need for a second edition confirms that sports medicine is not exclusively for elite athletes but serves every individual and the community, from childhood to old age. Knowledge of advances in sports medicine is essential for a broad range of professionals, including medical and healthcare workers, sports professionals, kinesiologists, coaches, and, of course, athletes and recreational sports enthusiasts. The book features contributions from 68 authors specializing in various fields, reflecting the interdisciplinary nature of sports medicine, which integrates knowledge from multiple disciplines in both scientific research and everyday practice. This interdisciplinarity remains the greatest strength of this textbook, which spans 511 pages and includes over 450 images and illustrations, along with more than 500 references.

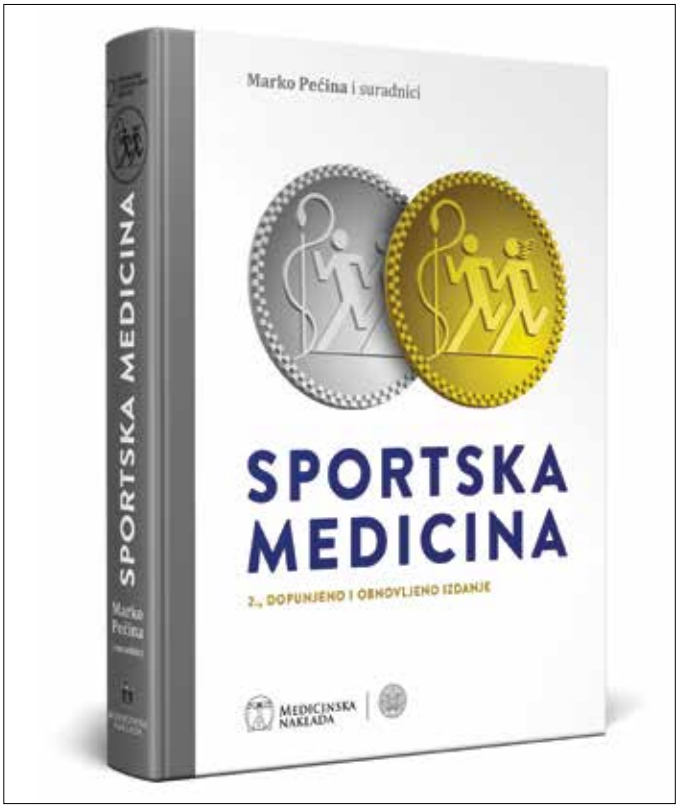
To provide readers with the best insight into the book, we present excerpts from the reviews of this second edition.

The first reviewer, Prof. Dr. Boris Labar, states:

"In addition to the chapters from the previous edition, appropriate updates have been made to the text and images. The textbook also includes seven new chapters: Overtraining Syndrome; Changes in Hematological and Biochemical Parameters in Athletes; Preserving Mental Health in Professional Athletes; Rotator Cuff Injuries of the Shoulder; Hip Impingement Syndrome; The Use of Orthoses in Sports; and Para-Athletes. This textbook is intended for a wide audience, from students to doctors, kinesiologists, physiotherapists, coaches, athletes, and recreational sports enthusiasts. It will also benefit the general public, as sports and recreation are an integral part of modern civilized society."

Reviewer Prof. Dr. Nebojša Popović adds:

"The textbook Sports Medicine is an example of how collaboration between experts from different fields of medicine can systematically and thoroughly address such a complex topic as sports medicine, or as the lead author calls it, 'the medicine of sport.' In the next edition of the textbook, some parts will need to be shortened or omitted. I strongly believe that artificial intelligence will play a role in this process. I am also confident that future editions will describe robotic surgical techniques in some chapters."



In his review, Prof. Dr. Domagoj Delimar states:

"The textbook covers all aspects of sports medicine, presenting them based on the experience of the lead author as well as numerous co-authors from various specialized fields. The co-authors are university professors from the Faculty of Medicine and the Faculty of Kinesiology at the University of Zagreb. The book's precision, systematic approach, logical structure, and progression make it fully comprehensible and easy to read. We recognize the challenges of writing a textbook on such a broad subject as sports medicine (the medicine of sport); however, we hope that this second, supplemented, and revised edition will contribute not only to the development of sports medicine but also to physical culture and sports in general in our country. The lead author and contributing writers have strived to provide the most comprehensive understanding of modern knowledge in their respective areas."

Publisher: Medicinska naklada, Zagreb
Year of Publication: 2025
Format: 21 x 30 cm
Binding: Hardcover
Extent: 511 pages, 46 chapters

8th Scientific Symposium "Josip Matovinovic" titled "Thyroid in Health and Disease", November 29, 2024, the Library Hall of the Croatian Academy of Sciences and Arts

Authors:
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On November 29, 2024, the 8th Scientific Symposium "Josip Matovinović", titled **Thyroid in Health and Disease**, was held in the Library Hall of the Croatian Academy of Sciences and Arts in Zagreb.

The Symposium was organized by the Croatian Thyroid Society, Department of Oncology and Nuclear Medicine, Sestre Milosrdnice University Hospital Centre, Zagreb – Centre of Reference for Thyroid Diseases of the Ministry of Health, and Medical Class of the Croatia Academy of Sciences and Arts. More than 100 participants attended the Symposium. Academician Zvonko Kusić, the President of the Croatian Thyroid Society and President of the Organizing Committee held a welcome speech as the introduction to the Symposium, followed by the greeting of the academician Vida Demarin in front of the Medical Class of the Croatian Academy of Sciences and Arts. Welcome speeches also held Professor Tomislav Jukić as the Vice President of the Organizing Committee and Verica Mešić, the President of the Croatian Thyroid Patients Association. In the first part of the Symposium, renowned Croatian experts and scientists presented the latest approaches in the surgical treatment, ultrasound diagnostics, histopathological diagnosis, iodine-131 therapy, and oncological treatment and monitoring of malignant thyroid tumors. Croatia has a high incidence of thyroid cancer. According to the published data from the Croatian Cancer Registry (2020), the incidence of thyroid cancer in Croatia is 16.5 cases per 100,000 population annually, placing it among the top five countries in Europe. In recent years, there have been changes in the treatment of differentiated thyroid cancers, marked by a less aggressive approach, especially for smaller tumors confined to the thyroid. Moreover, the development and introduction of new drugs for the treatment of poorly differentiated thyroid cancers have significantly improved the prognosis for these patients. We are witnessing the development of personalized medicine, with the possibility of comprehensive genetic profiling of tumors and targeted treatment for our patients. Therefore, the primary focus was on new treatment approaches, as well as recent changes in the histopathological diagnosis of thyroid tumors. Professor Drago Prgomet and Associate Professor Alan Pegan presented the latest approaches in surgical treatment of thyroid cancer. Vedrana Gladić Nenadić, MD, PhD presented ultrasound features of thyroid cancer. Professor Sanja Kusačić Kuna presented the latest approaches in radioiodine treatment of thyroid cancer. Associate professor Bernardica

Academician Zvonko Kusić, the President of the Organizing Committee, greetings the participants.

Jurić presented the recent histopathological classification of thyroid tumors. Nina Dabelić, MD. PhD, presented advances in oncological treatment of thyroid cancer and Professor Ivan Mihaljević and Professor Ante Punda presented up to date follow up strategy for differentiated and medullary thyroid cancer.

The second part of the Symposium was dedicated to the thyroid and its impact on various organs and organ systems, continuing the theme addressed at last year's Symposium. Thyroid hormones affect numerous organs and systems, being especially crucial for growth and development, primarily of the brain and skeletal system in fetuses and young children. A deficiency of thyroid hormones from birth leads to psychomotor retardation. Thyroid dysfunctions and thyroid diseases are common in the population, particularly affecting women. Thyroid disorders in adults have significant consequences, mainly affecting the cardiovascular, skeletal, central nervous, and other systems and organs. In this part of the Symposium, academician Bojan Jelaković presented the results of the EHUH 2 project. The project investigated the prevalence of hypertension, cardiovascular diseases, thyroid disorders, as well as the intake of salt, potassium, and iodine in different regions of Croatia. The ultimate goal of the project was primary prevention of cardiovascular diseases through the timely detection and treatment of hypertension and reduction of salt intake in our diet. Adequate iodine intake, which is an essential component of thyroid hormones, plays a crucial role in thyroid function. Our country, especially the Department of Oncology and Nuclear Medicine, Sestre Milosrdnice University Hospital Centre has extensive and successful experience in controlling goiter through adequate iodine prophylaxis, leading to the elimination of goiter and other disorders caused by iodine deficiency, the most important of which is psychomotor developmental delay. This achievement is of immense value to any population, and as a result, Croatia is among the countries that have most successfully addressed this important public health issue. Our salt is iodized, and with the reduction of salt intake, iodine intake will decrease as well, making continuous monitoring essential.

The Symposium also addressed the impact of chronic kidney disease on the thyroid, lecture held by Associate Professor Josipa Josipović as well as psychiatric aspects of thyroid disorders presented by Professor Dalibor Karlović.

Professor Drago Prgomet gives a lecture: "New approaches in the surgery of thyroid cancers confined to the thyroid". The Chairman of this session were Professor Dražen Huić and Professor Maja Franceschi.

CROVASCULAR 2025 - 10th Croatian Vascular Day: A Decade of Advancing Vascular Medicine in Croatia

Authors:
Mislav Vrsalović
Vida Demarin

Figure 1. Prof Krešimir Luetić, prof. Aleš Blinc, prof. Šekib Sokolović, prof. Diana Delić-Brkljačić, acad. Vida Demarin, prof. Mislav Vrsalović, acad. Zvonko Kusić, prof. Davor Vagić, prof. Miljenko Kovačević (from left to right) during 10th Croatian Symposium on Vascular Medicine - CROVASCULAR 2025.

The 10th traditional scientific symposium dedicated to the field of vascular medicine, Croatian Vascular Day - CROVASCULAR 2025 was held on 14 February 2025, under the auspices of the Department of Medical Sciences of the Croatian Academy of Sciences and Arts, the Sisters of Charity University Hospital Centre, the Croatian Cardiac Society, and the Croatian Society of Angiology of the Croatian Medical Association.

The symposium "CROVASCULAR - Croatian Vascular Day" was established in 2015 as a unique professional and scientific event in Croatia covering all aspects of vascular diseases. It was conceived as a space for interdisciplinary exchange, collaboration and education, bringing together cardiologists, vascular surgeons, angiologists, interventional radiologists, neurologists and general practitioners. Over the years, the event has developed into a highly respected scientific platform for the exchange of knowledge and clinical expertise in vascular medicine (angiology), promoting interdisciplinarity, teamwork and a holistic, evidence-based and personalised approach to the vascular patient. This foundation is crucial for professional and scientific progress, which ultimately leads to the successful treatment of often underestimated vascular diseases. The symposium traditionally included faculty and clinicians from the medical schools of the Universities of Zagreb, Split, Rijeka and Osijek, the Catholic University of Croatia as well as Croatian clinical centres and clinical hospitals together with international guests.

The event is accredited by the Croatian Medical Chamber and remains free of registration fees in order to uphold the principle of open access to expertise and scientific knowledge in cardiovascular medicine.

At the forefront of this event is its founder and driving force Prof Mislav Vrsalović, a renowned figure in vascular medicine at national and European level. Prof Vrsalović, the president of the symposium's organising committee, is an associate member of the Croatian Academy of Sciences and Arts (HAZU), president of the Croatian Society of Angiology of the Croatian Medical Association, head of the Department of Vascular Diseases and Hypertension at the Cardiovascular Diseases Clinic of the Sisters of Charity University Hospital Centre and head of the Ministry of Health's

Figure 2. Prof Vrsalović giving a talk at 10th Croatian Symposium on Vascular Medicine - CROVASCULAR 2025.

Referral Centre for Peripheral and Multisite Artery Disease. He is also active internationally, including as a board member of the European Society of Vascular Medicine (ESVM), as a nucleus member and treasurer of the European Society of Cardiology (ESC) Working Group on Aorta and Peripheral Vascular Diseases, and as a national representative in the Division of Angiology of the European Union of Medical Specialists (UEMS). Prof Vrsalović is both a Fellow of the European Society of Cardiology (FESC) and a Fellow of the Society of Vascular Medicine (USA).

The aim of this year's event was to present a rational diagnostic approach and optimal treatment options for vascular diseases from an interdisciplinary perspective. The focus was on raising awareness of the high morbidity and mortality associated with vascular diseases, as well as the chronic nature and frequent recurrence of these conditions. The limited public knowledge about these diseases was also addressed. A core mission of CROVASCULAR remains the promotion of sound diagnostics, optimal treatment strategies and public involvement through healthcare professionals and the media.

The symposium was divided into four structured sessions: Angiology Highlights, ABC of Angiology, Vascular Puzzle and Vascular Dilemmas, in which uncertainties in the treatment of vascular patients were explored using case studies from clinical practise, all in the light of the latest European guidelines. The programme was rich in content and interdisciplinary in nature.

The lectures addressed the epidemiology of vascular diseases and non-traumatic limb amputations in Croatia, a rational clinical approach to peripheral and polyvascular arterial disease in the presence of comorbidities (diabetes, chronic kidney disease, heart failure) and the prognostic role of carotid and femoral ultrasound in the assessment of cardiovascular risk. The modern methods of interventional endovascular treatment, including intravascular ultrasound, lithotripsy and rotablation in peripheral arterial disease, were also discussed. In collaboration with the Croatian Society for Vascular Surgery, topics on thoracic and abdominal aortic aneurysms, infectious aortitis and endovascular treatment of the aorta were discussed. Other presentations dealt with the epidemiology and risk stratification of pulmonary embolism, chronic venous insufficiency and the endovascular treatment of pulmonary embolism using mechanical thrombectomy in Croatian vascular centres. A large part of the event focused on complex cases and practical challenges in daily clinical practice. In the final block, "Vascular Dilemmas"," complex clinical cases such as carotid artery dissection in pregnancy and the management of Paget-Schroetter disease were presented.

Vascular diseases are common in the general population and are associated with a high mortality rate. They tend to recur, are chronic, and are relatively little recognised by the public. Therefore, the aim of this tenth traditional vascular symposium was to increase awareness of these diseases in the medical community and subsequently, via healthcare professionals and the media, in the general population.

Over the past ten years, CROVASCULAR has become a nationally and regionally recognised event that combines clinical excellence with scientific innovation. The symposium has been instrumental in raising awareness of vascular diseases in Croatia, which despite their high morbidity and mortality are still under-recognised and under-treated. It supports a holistic and patient-centred approach and has played a key role in establishing standards of care, including the establishment of the first Croatian Peripheral Arterial Disease Registry (CRO-PAD) in 2010 at the Sisters of Charity University Hospital Centre. This prospective, single-centre database has contributed significantly in improving vascular outcomes and patient care (1-10).

Looking to the future, the success of the 10th edition of CROVASCULAR stands as a testament to the sustained commitment of the organisers and participants. Thanks to their vision and dedication, the symposium has become a vital part of the country's medical landscape, fostering innovation, interdisciplinary collaboration and improved patient outcomes. It not only reflects a decade of achievements in Croatian vascular medicine, but also sets the course for future progress and innovation in the field.

The symposium was officially opened with a welcoming speech by Acad. Vida Demarin, Secretary of the Department of Medical Sciences at HAZU, Prof Davor Vagić, Director of the Sisters of Charity University Hospital Centre, Asst. Prof Krešimir Luetić,

Figure 3. Croatian Symposia on Vascular Medicine CROVASCULAR 2015-2025.

President of the Croatian Medical Chamber, and Prof Diana Delić-Brkljačić, Vice President of the Croatian Cardiac Society. With enthusiasm building towards CROVASCULAR 2026 and beyond, this anniversary edition is both a celebration and a call for further progress and action to address and reduce the silent but deadly burden of vascular diseases in Croatia.

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Clinical psycho-neuro-endocrine-immunology in practice (PNEI)

Author:
Sanja Toljan

The 6th Cours in Clinical PNEI, organized by Croatian Academy of Sciences and Arts, Department of Medical Sciences and Orlando Clinic form Zagreb, was held form April 22th – April 25th, 2025. in Zagreb. Participants came from different medical backgrounds, but with the same goal: to study clinical PNEI and to implement it in their everyday medical practice.

Clinical PNEI is a relatively new medical branch that has been rarely presented to caregivers, which makes this course very attractive for participants. The paradigm shift was brought through "new lenses" onto "old medical postulates". There is still ultimate challenge to implement new pathophysiology into understanding the nature of diseases and new discoveries about treatment, not only pharmacological. All teachings are provided with solid evidence-based support.

The lecturers were medical doctors, but also nutritionists, pharmacists, psychologists and kinesiologists, and the themes that were brought to participants were:

Introduction to Clinical PNEI, Physiology and pathophysiology in clinical PNEI, Diagnostics in PNEI, Therapeutic goals in PNEI, Neuroplasticity, Circadian rhythm and its clinical importance, Microbiome in clinical practice, Stress management strategies in PNEI, Pharmacotherapy, Anti-inflammatory diet, Physical activity in PNEI, Hormone replacement therapy.

The course also offered case reports, which encouraged participants to take active role in the implementation of PNEI approach.

Participants spent most of the time together, eating together healthy lunches, as a reminder of healthy practice, not only for patients, but also for people who provide medical care. "Medice, cura te ipsum",

is strongly advised during the course. Medical knowledge taught in Course, is strongly advised to caregivers to heal themselves, and as healthy persons, easily can provide authentic medical care to complicated and complex pathologies.
The Course offered new medical approach, changing of a paradigm in health care to experienced participants, but also to young doctors. Participants are encouraged to continue their education in PNEI by following the medical databases, as the knowledge in this discipline is spreading very quickly, offering new therapeutic solutions.

3rd International andrology symposium "News in andrology"

Author:
Dinko Hauptman

The Department of Medical Sciences of the Croatian Academy of Sciences and Arts, in collaboration with the Croatian Andrological Society of the Croatian Medical Association, successfully organized the 3rd International Symposium: News in Andrology. The event was held at the Library Hall of the Croatian Academy of Sciences and Arts in Zagreb on Friday, April 25, 2025, under the chairmanship of Academician Željko Kaštelan, the president of Croatian Society of Andrology. This one-day scientific gathering brought together leading experts from Croatia, Hungary, and Serbia, serving as a crucial platform for the exchange of the latest scientific and clinical knowledge in the dynamic field of andrology.

The symposium opened with welcoming remarks from Academician Josip Madić, deputy secretary, Department of medical sciences and Academician Željko Kaštelan, who emphasized the importance of continuous progress and collaboration in andrology. The morning session, moderated by Dinko Hauptman, Antun Gršković, and Pero Bokarica, focused on contemporary surgical and functional challenges. Presentations included Miodrag Aćimović's insights into the management of urethral strictures after radical prostatectomy, István Király's (University of Szeged) approach to treating severe Peyronie's disease with curvatures over 60 degrees, Radoš Đinović's discussion of complications and treatment following penile implant surgery, Dinko Hauptman's experience with penile implants, and Antun Gršković's analysis of quality of life following artificial urinary sphincter implantation for severe incontinence.

After a short break, the scientific program continued under the moderation of Davor Ježek, Igor Grubišić, and Damir Prlić, shifting attention to new diagnostic and therapeutic developments in male infertility. Miklós Szűcs (University of Debrecen) examined the impact of sperm DNA fragmentation on the interpretation of classical sperm parameters, offering new perspectives for infertility diagnostics. András Balló (University of Pécs) discussed the

importance of patient education in sperm retrieval and IVF, highlighting its role in optimizing clinical outcomes. Lana Škrgatić presented on IVF/ICSI after TESE/mTESE, while Domagoj Rašić compared fresh versus cryopreserved mTESE procedures. Igor Grubišić evaluated the clinical relevance of new genetic markers for infertility, and Zoran Zimak addressed updated protocols and outcomes in the treatment of varicocele and azoospermia.

The symposium highlighted the pivotal role of the Croatian Andrological Society, established in 2016, in promoting andrology as a multidisciplinary subspecialty within Croatian medicine. The society actively fosters scientific exchange, educates young physicians, and contributes to the development of novel diagnostic and therapeutic methods. Notably, Croatian experts, through collaborations with the European Academy of Andrology and numerous international centers, have made significant contributions to reproductive medicine and andrology at a European level.

The establishment of the Center for Reproductive Medicine and Andrology at the University Hospital Centre Zagreb, which has introduced a comprehensive range of new diagnostic and therapeutic procedures, further underscores Croatia's commitment to advancing andrological care.

The 3rd International Symposium: News in Andrology facilitated invaluable discussions and knowledge sharing among experts from diverse medical disciplines. It fostered an environment conducive to establishing new collaborations, notably strengthening regional cooperation with participating experts from neighboring Hungary and Serbia. These international partnerships are paramount for the continued development of andrology and the overall improvement of men's health in Croatia. The symposium was accredited according to the regulations of the Croatian Medical Chamber, underscoring its recognized contribution to professional development and medical education.

Hunting the Silent Killer
A Comprehensive Public Health of the Croatian Hypertension League. Report on activities from December 2024 to May 2025

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The Croatian Hypertension League has continued all activities and efforts in raising awareness about health lifestyle and adherence in general population and patients, as well as in decreasing clinical inertia among health care workers. It combines medical excellence, strategic communication, and community-based outreach to address major risk factors of cardio-kidney-neuro-metabolic diseases and mortality, where hypertension, the top silent killer no.1 and dyslipidemia, severely undiagnosed and untreated risk factor, are in the focus. The long-lasting national public health campaign ***Hunting the Silent Killer*** has started in 2019 and has become the most recognizable and impactful public health action in Croatia with an echo in many European countries, as well as in overseas countries such as Australia and Brasile. This action is under high patronage of the ***Croatian Academy of Scientists and Arts***. The main aim of this action is to increase health literacy of general population, and to organize primary preventive examinations of population living in remote areas of Croatia which is lacking physicians and other healthcare workers. All educational activities are organized in a "classical" way on public places where citizens are examined and given educative materials and advice. The mobile team of the Croatian Hypertension League consists of leading experts in the field, nurses, pharmacists, kinesiologists, nutritionists, but also medical students from all schools of medicine in Croatia, school of pharmacy, kinesiology students and pupils from medical school for nurses. The reach of the campaign extended geographically—from Dubrovnik on the south of Adriatic coast to Rijeka at the North, and Vukovar and Osijek in the east; from Varaždin and Krapina in the North-West to the small village of Majur in the heart of Banija, the poorest and mostly forgotten part of Croatia. Our islands where health care in general is not at the level as it is in the continental part of Croatia are of special interest. Last four years we are organizing the *sub-hunt* named ***Health on island.*** In every location, the campaign met citizens where they were, both literally and figuratively, bringing healthcare into the heart of everyday life, in the footsteps of their homes. Each action provided citizens with the opportunity to check blood pressure, cholesterol and glucose levels, and body composition (metabolic scale), ECG, as well as to receive personalized consultations from medical professionals. In remote areas all cardio-kidney-metabolic risk factors are analyzed, and local physicians, or major when there are no health care workers, receive all results which can be used in regular clinic work. If someone needs additional examination and evaluation, Crotian Hypertension League organizes all further needed procedures either in local hospitals or in one of hospitals in Zagreb. Last year was very successful, and more than thirty educative public health actions were organized across Croatia and several thousand citizens were examined and educated. One action should be particularly underlined. This is the summer action, a part of sub-hunt *Health on islands,* when a group of medical and pharmacy students and nurses went to the city of Benkovac instead of planned island Dugi otok when we heard that this city is lacking family physicians. During the five days, five hundred citizens of this small Dalmatian city were completely examined. The other arm for general population of the action is organized through the *digital cosmos* of the Croatian Hypertension League - web platform, newsletters, social networks. The other aim of our action, as already mentioned, is to increase the health literacy of health-care workers trying to improve knowledge and to decrease clinical inertia. This part also has two arms – the first one is "classical" face-to-face seminars and conferences. Here we must underline ***School of communication in hypertension.*** Education on how to communicate is still lacking in most of curriculum, and Croatian Hypertension League is trying to overcome this drawback, since good communication is the first and one of crucial steps to better disease control. The other arm here is digital as well and is organized via ***HealthMed*** educative web platform which is the most comprehensive medical digital educative site in Croatia. Every month two academic educative webinars are organized by key opinion leaders in cardio-kidney-neuro-metabolic diseases and two educative newsletters are sent to more than 10.000 healthcare workers.

The end of 2024 was also impressive when Croatian Hypertension League and *Hunting the Silent Killer* joined forces with the Zagreb Advent Run, Europe's largest costumed charity race. As a key partner of the event, the campaign reached a broad and diverse audience, embedding its health message into a celebratory, high-energy atmosphere drawing attention to lifestyle-related risk factors such as sedentary lifestyle, physical inactivity, high salt intake, and insufficient intake of vegetables and fruits.

In 2025, the activities continued with the same enthusiasm. Giving more emphasize on hypertension and dyslipidemia, in 2023 we have stared two sister sub-actions of the main *Hunting the silent killer* program. The first one is ***Mission 70/26*** which aim is that till 2026 70% of hypertensive patients treated be under control, and the other one ***What is your number?.*** has the aim to increase awareness about dyslipidemia i.e. hypercholesterolemia. Both campaigns support the core mission of empowering citizens to take ownership of their cardio-kidney-neuro-metabolic health, while also fostering accountability within the healthcare system. The first action in 2025 was organized in the city of Split when the whole **University of Split** officially became a member and partner of the Croatian Hypertension League. In Slavonski Brod,

the campaign aligned itself with an extraordinary humanitarian effort when at the Poloj Ultra marathon. Marko Kos, Croatian athlete and ambassador of *Hunting the Silent Killer*, broke a Guinness World Record by running 100 kilometers while pushing five children with disabilities in a specially adapted racing wheelchair. To increase awareness on obesity and chronic kidney disease at the World Obesity Day and World Kidney Day several public health actions were organized in the shopping centers in Zagreb, Osijek and Split. During the *Zagorje Days of the Croatian Hypertension League* public health action was organized in the city of Krapina and later in the city of Zlatar. On the way back to Zagreb we organized an action in Stenjevac, a suburban part of Zagreb. Campaign's spirit of engagement also flourished at the Rijeka Carnival, where the message of health promotion was delivered through creative expression and playful symbolism. Masks representing modern health threats, demons as we call them in the Croatian Hypertension league, high salt intake, sedentary lifestyle, obesity, smoking, not enough consummation of vegetables and fruits, incorrect blood pressure measurements, poor adherence, but also climate changes, air pollution and stress were "defeated" in a symbolic, festive procession. The event emphasized collective responsibility and reinforced the idea that public health is not just about individual decisions but also about social cohesion and shared values. Complementing these efforts, the campaign ensured that medically underserved and aging populations in more remote villages such as Majur or islands were not left behind. In these communities, detailed health assessments including ECG, central blood pressure measurements, arterial stiffness, ultrasound examination, and biochemical tests of all cardio-kidney-metabolic variables offered potentially life-saving insight, often for the first time in life. In April we organized an action during the symposium on Chronic non-communicable diseases in Zagreb, and during the symposium of obesity which was held in the city of Opatija we organized another action when we launched a public bus marked with signs of our action. Youth engagement remained a strategic pillar of the action. The second annual race, *Trk! Za zdravlje* ("Run! For Health"), was organized in Zagreb. This is a children's race designed to encourage healthy habits from an early age. Hereby we used sport as a medium for education and empowerment. Parallel to field actions, the campaign made a strong visual statement across public spaces. After cities of Varaždin, Osijek, Rijeka and Krapina this year city of Opatija joined the action by coloring public buses with educative messages and signs of our action. Furthermore, large-format billboards featuring campaign ambassadors—Tatjana Matejaš Cameron (Tajči), Juraj Šebalj, Tomislav Bralić, and Nikša Kaleb—appeared throughout Zagreb, Šibenik, Split, Osijek, Opatija, Rijeka and many other places. Their message was clear and urgent: Do not ignore warning signs! Get checked! Take responsibility for your health! These familiar and famous faces helped expand the campaign's reach to broader segments of the population, including those less likely to interact with conventional medical messaging. Looking ahead, the month of May—globally recognized as Hypertension Awareness Month—will bring at least five additional actions across Croatia, culminating in World Hypertension Day. These events will reaffirm the League's enduring commitment to prevention, early diagnosis, and citizen empowerment. We will inform you about this action and all other things planned for this year in our next report. All these activities have the root in the EH-UH 1 study which started in 2000. From this long-ago time our motto was and will remain the one from William the Silent: ***One need not hope in order to undertake, nor succeed in order to persevere.*** In this case we completely disagree with the famous Edmont Dantes´ quotation: All human wisdom is contained in these two words - Wait and Hope.

Through its holistic, inclusive, and scientifically grounded approach, ***Hunting the Silent Killer*** has become a model of how a national campaign can engage diverse communities, overcome healthcare disparities, and promote lasting behavioral change. The Croatian Hypertension League has demonstrated that public health, when delivered with compassion, creativity, and consistency, can become a shared national mission.

With *Hunt targeting the silent killer(s)*, saving lives has never been easier. Do not hesitate! Join us! Become a hunter for your health and truly happiness.

35th SUMMER STROKE SCHOOL "HEALTHY LIFESTYLE AND PREVENTION OF STROKE AND OTHER BRAIN IMPAIRMENTS"- Dubrovnik, June 2nd - 6th 2025.

Authors:
Vida Demarin
Hrvoje Budinčević

Our traditional 35th Summer Stroke School, "Healthy Lifestyle and Prevention of Stroke and Other Brain Impairments," was held at the Inter-University Center (IUC) in Dubrovnik.

The Summer Stroke School, part of the academic program of Inter-University Centre Dubrovnik, was organized by the International Institute for Brain Health and Croatian Stroke Society and co-organized by the Department of Medical Sciences of the Croatian Academy of Sciences and Arts, Central and Eastern European Stroke Society, and from this year with Croatian Neurological Society – Intensive Neurology Section. It was accredited with 4 ECTS credits by the Croatian Medical Chamber.

Prof. Vida Demarin, the founder and course director, successfully ran the school with the board of directors, Prof. Kurt Niederkorn, and Prof. Yoshikazu Yonei. Prof. Marina Roje Bedeković, and Assist. Prof. Hrvoje Budincevic. In addition to the Course Directors, faculty this year's school included experts and excellent lecturers.

The school's program, with its rich tradition of inspiring and knowledge-rich content, attracted participants from a diverse range of countries, primarily from Central and Eastern European countries. This international participation underscores the global impact of our event.

The main themes included lectures and discussions on the current management of ischemic and hemorrhagic stroke (stroke prevention, diagnostics, and treatment), headache/migraine, multiple sclerosis, and neurodegenerative diseases such as Parkinson's and Alzheimer's disease. We organized the cerebrovascular ultrasound hands-on workshops again.

During this interesting program, participants were very satisfied with its content and the opportunity to discuss in a friendly atmosphere with lecturers and to try and practice ultrasound examination during a Cerebrovascular Ultrasound hands-on workshop with an individual approach.

During the IUC Welcome Reception, Prof. Vida Demarin, Prof. Kurt Niederkorn, and Assistant Prof. Hrvoje Budinčević were awarded by the IUC for their outstanding dedication to organizing and participating in the Summer Stroke School series, thereby enriching the IUC academic environment.

We eagerly look forward to welcoming you next year in Dubrovnik from June 1st to 5th, 2026. Your presence is crucial to the success of our event, and we value your contribution to our academic community.

MIND & BRAIN – 64rd International Neuropsychiatric Congress, Pula, Croatia – 29th May – 1st June 2025

Authors:
Vida Demarin
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The Mind&Brain - 64th International Neuropsychiatric Congress was held in Pula from May 29 to June 1, 2025.

The Congress was organized by the International Institute for Brain Health and the Department of Medical Sciences of the Croatian Academy of Sciences and Arts, together with the Society for Neuropsychiatry, Department of Neurology – University Hospital Center "Sestre milosrdnice", Croatian Society for Personology, Personality and Eating Disorders, Central and Eastern European Stroke Society (CEESS) and Croatian Stroke Society under endorsement of the World Federation of Neurology (WFN) and European Academy of Neurology (EAN). The Croatian Medical Chamber, Croatian Nursing Council, and Croatian Council of Physiotherapists accredited the Congress. Congress president Prof. Vida Demarin led the Congress. This year, the President of the Organizing Committee was Prof. Marina Roje Bedeković.

Thanks to our consistently outstanding scientific program, which bridges neurology and psychiatry and adjacent disciplines, we saw a diverse group of 300 registered participants. Notably, our program continues to attract interest from beyond Central European countries, with participants joining us from as far as South Korea.

The first day of the Congress was reserved for registration and networking activities, as well as scientific symposia on multiple sclerosis, dementia, and dysphagia. The scientific program, held on May 30th and 31st, commenced with Plenary sessions that included interdisciplinary lectures in neurology and psychiatry. After the Plenary session the Congress was divided into two or three parallel sessions on following topics: Graz Stroke Symposium (Management of subarachnoid hemorrhage), Neuropsychiatry Symposium (Overlapping mechanisms in neurodegeneration and mental health disorders), Psychiatry Symposium (Personality development and pathology in an evolutionary perspective; Psychiatry in neurology, Psychiatry in neurology), 2nd symposium on neurodevelopment disorders, Central and Eastern European Stroke Society (CEESS) Stroke Symposium, Neurodegenerative Symposium, Headache Symposium, Psychoneuroendocrinoimmunology Symposium on approach to kynurenine pathway, Epilepsy Symposium, HISPA & NAM Symposium, Neurodegenerative and Neuropsychiatry Symposium, and Selected Oral Presentations Symposium. We organized a traditional Psychopathology Summer School on novel approaches to schizophrenia research and practice.

The poster session involved 56 poster presentations. Prizes were awarded to the three best posters from neurology and the three from psychiatry. The City of Graz provided the first two prizes, and INPC Kuratorium and the International Institute for Brain Health provided the second and third prizes, respectively. Those posters were presented at a special session (Best Posters' Awards Presentation Session)

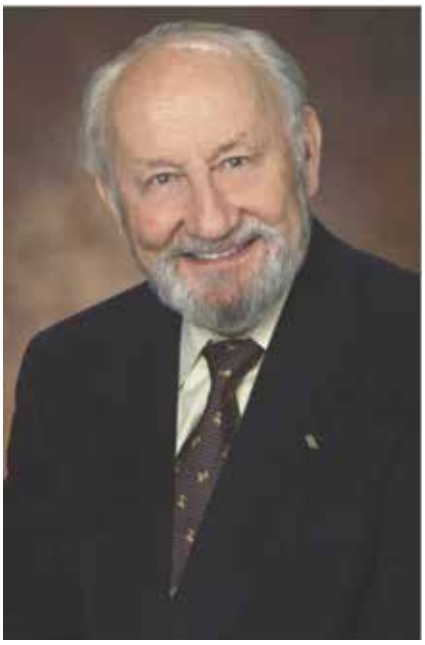
The Satellite Symposia were rich in recent data on the latest therapeutic possibilities and achievements. The last day was reserved for meetings of the Kuratorium, Central and Eastern European Stroke Society, and International Institute for Brain Health.

Over these four days, our Mind & Brain Congress was a resounding success, fostering numerous interactions and lively discussions. Distinguished lecturers generously shared their vast knowledge and expertise with their younger colleagues, thereby broadening perspectives and paving the way for new directions. This collaborative spirit is at the heart of our Congress, embodying the founding idea of the Pula School of Sciences and Humanities, which was particularly evident during the Posters' Awards Presentation Session.

We look forward to another exciting opportunity to learn, share, and grow together next year in May!

In-memoriam: Stevo Julius

Author:
Bojan Jelaković



Stevo Julius graduated from the Faculty of Medicine, University of Zagreb. In 1965, he joined the faculty of the University of Michigan, Ann Arbor , where he spent his working life, working almost until the last years of his life as an active retired (emeritus) professor of medicine and physiology. His research contributions in the field of hypertension and contributions to education on arterial hypertension, earned Stevo Julius an international reputation. He always proudly emphasized his ties to the homeland that educated him and from which he set out on his life's adventure.

Stevo Julius was born in 1929 in Kovin, on the banks of the Danube in Serbia, where he lived until he was eleven years old, when he moved with his family to Zagreb. He spent his childhood and youth in Zagreb, but the chaos of World War II also caught up with him there. He described that period, and the following year, in his book "Neither Red nor Dead", the Croatian edition of which was presented to the public at the Faculty of Medicine of the University of Zagreb. Stevo Julius graduated from high school in 1947 in Zagreb and the same year enrolled at the Faculty of Medicine of the University of Zagreb, graduating in 1953. Both the years after the war and after graduation were turbulent for Stevo Julius, because he spent the initial part of his working life in Goražde , according to the decree. He finally began to build his professional and scientific career at the Clinical Hospital Center Zagreb, at Rebro, and at the Faculty of Medicine of the University of Zagreb, from where he left for his first stay in the United States of America, in Ann Arbor , in 1962 . Because of the agreement that existed between the United States of America and Yugoslavia, he returned to Zagreb again, completed his specialization and received his doctorate. Due to the excellent impression he left in Ann Arbor, he received an invitation and an offer he could not refuse, and since 1965 he has been continuing his career and life in Ann Arbor .

In his prolific scientific work, Stevo Julius has published more than 330 papers, book chapters and written several books. Some of his works are fundamental works for the understanding of arterial hypertension and enter the history of medicine along with Gyton , Laragh , Pickering , Pagae , Kaplan and other stalwarts of hypertension .

While still in Zagreb, Professor Julius showed an interest in understanding arterial hypertension, especially the role of the autonomic nervous system. His dissertation from 1962 was dedicated to this topic, as the title itself clearly states: "Psychosomatic characteristics of students with borderline high blood pressure".

Julius devoted the largest period of his research to the early phase of arterial hypertension, sometimes called borderline hypertension. Very early on, he noticed that there is a connection between the mechanisms that lead to the onset and maintenance of arterial hypertension, and a greater tendency to obesity in hypertensive patients, which he described excellently in one of his articles - "Hypertension and Obesity - a Two-Way Street". One could say that the crown of his research is the Tecumseh study, which is actually a small Framingham . It is smaller in terms of the number of subjects included and the duration of monitoring, but it produced some results that the Framingham study did not. This study is visionary and far ahead of its time in terms of its design because it is a blatant example and represents a model for how translational research in medicine should be organized. One of the key dilemmas that Professor Julius tried to resolve is how initial borderline hypertension transitions into permanent arterial hypertension, i.e. how the state of initially increased cardiac output transitions into a state of increased peripheral resistance.

Stevo Julius was involved in almost all the seminal interventional studies that strongly influenced today's guidelines such as HOT, LIFE and VALUE. All the time, he was thinking about whether it is possible to stop or at least delay the transition from the initial phase to stable arterial hypertension by short-term use of antihypertensive drugs . He realized that part of his dream in the TROPHY study, where he proved it. This revolutionary study

confirmed that this concept is correct and possible, and opened the door to a whole series of research that will probably answer the question of which of these patients in the initial phase of arterial hypertension, i.e. in the phase of prehypertension should be treated in this way.

Stevo Julius has consistently participated in all major conferences organized by the Croatian Hypertension Society, of which he is an honorary member. Since 2006, he has been a visiting professor at the Faculty of Medicine, University of Zagreb, and on his initiative, a cooperation agreement was signed in 2010 between the University of Ann Arbor and the Faculty of Medicine in Zagreb, opening the doors to numerous young Croatian experts and scientists.

He described his scientific life path in his book "Adventures in Hypertension", which was translated by the Croatian Hypertension Society and a Croatian edition is available. During Stevo's last stay, we were discussing a second edition, which we will now unfortunately have to do without him. That is a book that should be read by anyone who is in any way involved in arterial hypertension, medicine, or science. But it is also a book that can teach everyone important things in life. How to persevere, believe, learn, respect others, and always keep your spirit and sense of humor. Anyone who reads the book and goes through these adventures will be richer for a new experience, for a new journey.

As a sign of gratitude for all his achievements, for his tireless emphasis on where he came from, connecting it with his personal life success, the Croatian Society of Hypertension and the Croatian League of Hypertension organized a major international conference in 2022 called the "Stevo Julius Conference ", which was attended by leading experts from all continents. It is not necessary to describe his feeling of happiness, which was no less than the feeling of happiness of those of us who loved him. And we will always love and remember him for his wisdom, knowledge, gentleness, friendship and especially warm sense of humor. Although we know that we will not meet on these meridians and parallels, Stevo will always be there as an inspiration and encouragement for better and more, and his voice and laughter will resonate in our hearts.

Stevo, accompanied by his son Nikola, is leaving Zagreb after the large international congress "Stevo Julius Conference ". The last photo of him in Zagreb at the Zagreb airport was taken in November 2022 by my son Ivan, who had a particularly close relationship with Stevo, who seemed to have a premonition that we would never see each other again.