Vol 61, No 4, December, 2022
UDK 61
ISSN 0365-4478 (Printed)
ISSN 1821-2794 (Online)
www.medfak.ni.ac.rs/amm

ACTA MEDICA MEDIANAE

Naučni časopis
Medicinskog fakulteta Univerziteta u Nišu i
Podružnice Srpskog lekarskog društva u Nišu

AMM

Scientific Journal of the University of Niš Faculty of Medicine
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http://www.medfak.ni.ac.rs/amm

Acta Medica Medianae (UDK 61; ISSN 0365-4478 štampana verzija; ISSN 1821-2794 elektronska verzija) je zvanični časopis Medicinskog fakulteta Univerziteta u Nišu i Podružnice Srpskog lekarskog društva u Nišu pod pokroviteljstvom Ministarstva za nauku i tehnološki razvoj Republike Srbije. Časopis izlazi četiri puta godišnje od 1962 godine. Izdavač je Medicinski fakultet Univerziteta u Nišu, Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija. Sadržaj i celokupan tekst časopisa dostupan je na sajtu Medicinskog fakulteta http://www.medfak.ni.ac.rs/amm. Uputstvo autorima se objavljuje u svakom broju, pri čemu je autor dužan da se pridržava navedenih uputstava prilikom predaje rukopisa.
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Kontakt adresa: Časopis Acta Medica Medianae, Medicinski fakultet, Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
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Tiraž 200 primeraka. Štampa: "Sven", Niš, Srbija.
Acta Medica Medianae je trenutno indeksirana na *Index Copernicus-u, Srpskom citatnom indeksu, DOAJ i EBSCO*

Acta Medica Medianae (UDK 61; ISSN 0365-4478 printed version; ISSN 1821-2794 online) is the official Journal of the University of Niš Faculty of Medicine and the Department of the Serbian Medical Society in Niš published with the help of the Ministry of Science and Technological Development of the Republic of Serbia. The Journal has been published four times a year since 1962. The publisher is the University of Niš Faculty of Medicine, Institutional address: dr Zoran Đinđić 81, 18000 Niš, Serbia. Table of contents and full texts of articles are available on the Institutional Home Page at http://www.medfak.ni.ac.rs/amm. Prices are subject to change. All subscriptions start with the first issue of the current year. For payment details contact the Secreteriat at acta@medfak.ni.ac.rs. Instructions for authors appear in every issue. Manuscripts accepted for publication are not returned to the author(s). *Acta Medica Medianae* retains the right for further distribution and printing of the articles.
Editorial correspodence: Journal Acta Medica Medianae, Faculty of Medicine, Dr Zoran Đinđić 81, 18000 Niš, Serbia.
Electronic submission of the papers: acta@medfak.ni.ac.rs, Phone: +381-18-4533001 lok. 113 fax. +381-18-4534336
Printed on acid-free paper; 200 issues. Press: "Sven", Niš, Serbia
Acta Medica Medianae is currently indexed in Index Copernicus, Serbian Citation Index, DOAJ and EBSCO

Naučni časopis Medicinskog fakulteta Univerziteta u Nišu i Podružnice Srpskog lekarskog društva u Nišu

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Vol 61, No 4, December, 2022
UDK 61 ISSN 0365-4478 (Printed version)
ISSN 1821-2794 (Online)
http://www.medfak.ni.ac.rs/amm

Autor slike na prednjoj stranici:
Petar Petrović

ACTA MEDICA MEDIANAE

Vol 61, No 4, December, 2022

Secretariat

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ACTA MEDICA MEDIANAE

Vol 61, No 4, December, 2022

www.medfak.ni.ac.rs/amm

ACTA MEDICA MEDIANAE

Vol 61, No 4, Decembar, 2022

Uredništvo

www.medfak.ni.ac.rs/amm

ACTA MEDICA MEDIANAE

Vol 61, No 4, Decembar, 2022

www.medfak.ni.ac.rs/amm

Original article **UDC: 616.89-008.48:[616.98:578.834**
doi:10.5633/amm.2022.0401

AVAILABILITY OF SPECIAL TREATMENTS AND SERVICES FOR INDIVIDUALS WITH AUTISM SPECTRUM DISORDERS DURING COVID-19 STATE OF EMERGENCY AND LOCKDOWN IN SERBIA

Aleksandra Stojanović[1], Miodrag Stanković[1,2]

Restrictive measures and major lifestyle changes were brought for entire population during the beginning of the COVID-19 pandemic, and for individuals with Autism Spectrum Disorder (ASD) and their caregivers as well. Specialized treatments, organized education, and other services intended for the ASD population ceased, which affected overall functioning of this population and their caregivers. ASD itself significantly affects lives of individuals with this disorder and their families, and these individuals are particularly sensitive to changes in routines and daily functioning. The aim of this study was to explore changes in different treatments and services availability for individuals with ASD in Serbia at the beginning of the pandemic and during the state of emergency, and its impact on their caregivers. Participants completed the "Autism and COVID-19" survey, which was designed for primary caregivers of individuals with ASD. A sample of 89 participants completed the survey. They were recruited from patient databases, the parents' association (NGOs), and the specialized schools. The study was conducted during the state of emergency in the country, from April 13 to 25, 2020. The survey revealed a complete absence of specialized treatments, lack of support, feelings of helplessness of the caregivers, which have become more prominent during emergency state. The pandemic reveals underdeveloped strategies to maintain support for this population, and the need for better predictions in the future to protect this vulnerable population and their caregivers.

Acta Medica Medianae 2022;61(4):05-11.

Key words: *ASD, caregivers, support, COVID-19, lockdown*

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Introduction

At the end of 2019, there was a rapid spread of new corona virus infection, and the disease caused by it was called COVID-19. The disease was characterized by symptoms of various organic systems, and high contagiousness followed by very rapid spread of the disease. On January 30, 2020, the World Health Organization declared the pandemic (1). In many countries, various restrictive measures were introduced in an attempt to prevent the spread of the disease, with demands to change people's behavior. Namely, in many countries, restrictions of movement, work from home, schooling from home came into force, which led to a significant change in previous life habits. (2). These restrictive measures, as well as COVID-19 disease itself, influenced significantly the psychological functioning of people, people's mental health, and previously existing mental illnesses (3).

Autism spectrum disorder (ASD) is a neurodevelopmental disorder recognized in early childhood, and it extends throughout the life of the individual, with the incidence higher than 1/100 (4). Main characteristics of ASD are difficulties in social communication and interaction, and the existence of restricted, repetitive behaviors, interests and specific response to sensory input (5). This is a population of people with special needs (6), and their caregivers constantly face challenges. One of the main characteristics of ASD is extreme resistance to any form of change. People with ASD usually have a strict daily routine; they are involved in working with specialized therapists, in specialized institutions. The beginning of the pandemic brought great changes in the lives of individuals, including people with ASD (especially during the state of emergency), had a huge impact on families with ASD members, and multiplied the challenges they face every day anyway.

However, at the beginning of the pandemic, and especially during the state of emergency, children with ASD usually stayed home with their family members, without contact with their therapists (7). Restrictive measures themselves could lead to an exacerbation of behavioral problems of people with ASD (8). In Serbia, there are several different services for individuals with ASD (9); however, they often face difficulties, such as the unavailability of specialized programs in all parts of the country (10). A state of emergency with a police lockdown was declared in Serbia on March 15, 2020 and imposed movement restrictions, shortening working hours of various institutions, schooling from home and the cessation of many services that had existed until then. Complete absence of work with therapists and previous daily routines could cause various changes in behavior and reactions of people with ASD. In addition, it could be expected for caregivers to experience lack of overall support and higher level of stress (11), and concerns about worsening of symptoms (12). In addition to the characteristics of the disorder itself, the main problems of individuals with ASD and their family members are stigmatization, lack of general public awareness and knowledge (13), lack of society acceptance, and low level of social support (14).

The aim of the study was to explore availability of treatments, service and overall support for families of individuals with ASD in Serbia during the COVID-19 pandemic beginning and the police lockdown.

Subjects and methods

Participants completed a modified Caregiver Needs Survey, earlier developed by Amy Daniels and the National Coordinators of the Southeast European Autism Network (SEAN), which is a part of the Global Autism Public Health Initiative for Autism Speaks (AS). Modifications consisted of changes that issues were directly linked to the pandemic situation and the state of emergency. The final questionnaire contained 50 questions in Serbian language, which could be filled online in 15-20 minutes (link: https://docs.google.com/forms/d/e/1FAIpQLSfI-ws_zjOF62gnvxO2_u2GMboJffwfOwpjwU5KlVND8Yt8g/viewform?usp=sf_link). Before completing the questionnaire, participants gave consent. The survey was intended for caregivers of individuals with a diagnosis of ASD. The study was conducted from April 13 to 25, 2020, during the state of emergency in the country. A total of 89 caregivers completed the questionnaire; 80.9% mothers, 16.9% fathers and 2.2% other relatives and foster parents. The average age of ASD individuals at the time of survey competition was 8.9 years (SD 4.5; min 2; max 22).

Results

The study included 89 participants, caregivers of individuals with ASD who were involved in different types of specialized treatments before the beginning of the pandemic. The majority of the participants were mothers (80.9%), with 16.9% of fathers and another 2.2% of other caregivers. Most of the guardians had secondary school and a university degree, while a smaller part of them completed only primary school, or had unfinished university education, and more than a university degree. The individuals with ASD were mostly males (79.8% vs. 20.2%): 24.7% of them were nonverbal, 14.6% used only one word, 29.2% used sentences of 2-3 words, while a smaller percentage used simple and complex sentences (Table 1).

Table 1. Demographic characteristics of the study participants

Caregiver's education level	No	%
1. Elementary school	2	2.2
2. High school	39	43.8
3. Unfinished university Education	8	9.0
4. University education	39	43.8
5. More than university Degree	1	1.1
Caregiver		
Mother	72	80.9
Father	15	16.9
Other	2	2.2
Gender of individual with ASD		
Male	71	79.8
Female	18	20.2
Language development level		
Language not developed	22	24.7
Uses only single words	13	14.6
Uses 2-3 words sentences	26	29.2
Uses 4+ words sentences	10	11.2
Uses complex sentences	18	20.2

Prior to the pandemic, individuals with ASD were involved in the different types of treatment and support; most of them in two or more types of support (Table 2).

At the onset of the pandemic and emergency state, most of the participants (77.5%) responded that their child was not receiving any form of standard developmental non-pharmacological treatments any longer; 22.4% of the surveyed population remained in some form of contact with single information source and services; some percentage of guardians who had contact with a service reported contact with a child psychiatrist, general practitioner, child teacher, while some of them sought help from the Internet. Most guardians turned to other parents of children with autism for help (39.3%) (Table 3).

However, 75.3% of the responders felt that it was important to have contact with the services that worked with their child during the pandemic and the state of emergency in the country (61.8% thought that it was very important, while 13.5% thought that it was "most important").

Most respondents agreed that during the pandemic and the state of emergency they felt helpless because they had a child with autism (completely agreed 42.7%; mostly agreed 13.5%) (Table 4).

During the pandemic and the state of emergency in the country, 25.9% of the surveyed population felt discriminated because they had a child with autism, 11.2% were neutral, while 63% of them did not have this experience.

When it comes to satisfaction with the overall support received by the families of children with ASD during the beginning of the pandemic and the state of emergency, the majority stated that they were unsatisfied (Table 5).

Table 2. Specialized treatments and services used by individuals with ASD before the beginning of the pandemic

Service	Number	%
Personal assistant	24	29.2
Behavioral therapist	13	14.6
Developmental Pediatrician	8	9.0
Pedagogue	7	7.9
Nutritionist	3	3.4
Psychiatrist	44	49.4
Psychologist	42	47.2
Defectologist	54	60.7
Speech therapist	45	50.6
Music therapy	22	24.7

Table 3. Contacts with services after beginning the pandemic and introducing the lockdown

Service	No	%
Internet counseling	2	11.1
General practitioner	3	16.7
Child's teacher	6	33.3
Parents of individuals with ASD	6	39.3
Child psychiatrist	3	16.7

Table 4. Feeling helplessness because of having child with ASD during the pandemic

Statement	No	%
Completely agree	38	42.7
Mostly agree	12	13.5
Neither agree nor disagree	19	21.3
Mostly don't agree	9	10.1
Completely disagree	11	12.4

Table 5. Overall satisfaction with support after the beginning of the pandemic and introducing lockdown

Statement	Number	%
Completely satisfied	15	16.9
Mostly satisfied	3	3.4
Not satisfied nor Dissatisfied	24	27
Mostly dissatisfied	6	6.7
Completely dissatisfied	41	46.1

Discussion

Our sample consisted of 80.9% of mothers, 16.9% of fathers and 2.2% of other relatives and foster parents. This sample is in accordance with data from the relevant literature, which indicates that mothers more frequently take care of children's needs (15-17). However, the number of fathers participating in our study as the main caregivers is not small. The reason for this can be the state of quarantine and lockdown, which brought many families together, and put the fathers in the position of the main caregiver (18).

The education of people with autism is organized in different ways in different regions of the world (6). There is a number of different treatments in Serbia, although they are not uniformly organized throughout the country, and there are many areas where very few or none is available (15). In our study, we included only caregivers who stated that they received a certain type of support, and most of them used multiple modalities of support, most often the services of speech therapists, special educators, psychologists (Table 2). However, with the beginning of the pandemic, and especially the introduction of a state of emergency and strict movement restrictions, parents found themselves in a situation where they spent time with children at home, and they were completely isolated from contact with therapists, which significantly disrupted their daily routines. This is common to many countries around the world, that the beginning of the pandemic brought a complete cessation of specialized services and education for individuals with ASD (18). Namely, due to the restrictive measures that were introduced in almost all countries, the education of these individuals had to be stopped, and they were left without personal contact with therapists and teachers (19). Almost 80% of guardians in our research stated that at the beginning of the pandemic and during the state of emergency, they did not have contact with any of the services, and most of them emphasized the importance of such contacts (75%). In our sample, the majority of individuals with ASD has low level of language and speech development, most children are almost unable to express their needs in words, which makes them completely dependent on caregivers help. It also makes their treatments and rehabilitation essential for overall functioning, and their cessation can greatly affect worsening of problematic behaviors and loss of skills acquired so far (20).

Analyzing the results, we noticed that in the group of guardians who had contact with some service (less than 20% of participants), contacts with doctors, teachers and parents of other children, searching for information on the Internet were dominant, while contacts with specialized therapists that were present before the pandemic were completely missing (Table 3). Interrupting the continuity of their training leads to potentially significant changes in their behavior, because they do not understand the reasons for interrupting daily routines and usual activities. Psychosocial interventions in individuals with ASD can improve specific skills such as social communication, joint attention, language, engagement in social group, and this can affect further development and social adaptation (19). The overall satisfaction with the support during the pandemic and the state of emergency is estimated as low among our respondents, with more than half of them completely dissatisfied with the support. They maintained contact with other parents of children with ASD, and they searched for advice online.

In several studies from developed countries, we found that different specialized therapist and child and adolescent psychiatrist offered online counseling, because it was not possible to see their patients (18, 21, 22). Caregivers in these studies were looking for a medical and social support in taking care of ASD individuals (23), and reducing stress and anxiety level (24) in changed conditions of the pandemic.

Feeling of helplessness during the pandemic and the state of emergency was experienced by 56.2% of the caregivers. This is more often than what we expect from parents of children diagnosed with ASD (17). Twenty-five point nine percent of parents felt discriminated for having a child with ASD in challenging conditions of the pandemic. We believe that complete cessation of contact with various services, and the experience of being left to themselves and excluded from regular types of support contribute to the experience of discrimination and helplessness of ASD caregivers. In addition, we believe that it is essential to develop various strategies that would enable the smooth operation of specialized services working with these children for future emergency situations, especially bearing in mind how important for them is the structured environment and continuity of work, and that they are essential for their overall functioning.

Conclusion

This survey was completed by 89 primary caregivers of individuals with ASD during the beginning of the COVID-19 pandemic and police lockdown in Serbia. The survey revealed almost total cessation of specialized treatments, low overall support, feelings of discrimination and helplessness. At the beginning of the pandemic, most caregivers of individuals with ASD were left without any kind of specialized support, and had to take care of the needs of the children on their own at home. We believe that in the future, it is necessary to develop strategies for all types of emergencies in order to protect and give adequate support for individuals with ASD and their families.

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Originalni rad **UDC: 616.89-008.48:[616.98:578.834**
doi:10.5633/amm.2022.0401

DOSTUPNOST SPECIJALIZOVANIH TRETMANA I USLUGA ZA OSOBE SA POREMEĆAJEM IZ SPEKTRA AUTIZMA TOKOM VANREDNOG STANJA UZROKOVANOG PANDEMIJOM VIRUSA COVID-19 U SRBIJI

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Početak pandemije virusa COVID-19 i restriktivne mere donele su velike promene načina života celokupnog stanovništva, pa i osoba sa poremećajem iz spektra autizma (PSA) i njihovih staratelja. Ukidanje specijalizovanih tretmana, organizovanog obrazovanja i drugih usluga namenjenih populaciji sa PSA, uticalo je na ukupno funkcionisanje ove populacije i njihovih staratelja. Sam PSA značajno utiče na život osoba sa ovim poremećajem i njihove porodice i čini ove osobe posebno osetljivim na promene u rutini i svakodnevnom funkcionisanju. Cilj ovog istraživanja je da ispita promene u dostupnosti različitih tretmana i usluga za osobe sa PSA u Srbiji na početku pandemije virusa COVID-19 i tokom vanrednog stanja, kao i uticaj ovih promena na njihove staratelje. Učesnici su popunili upitnik „Autizam i COVID-19", koji je dizajniran za primarne staratelje osoba sa PSA. Osamdeset devet ispitanika učestvovalo je u ovom istraživanju. Istraživanje je sprovedeno tokom vanrednog stanja u Srbiji. Rezultati su pokazali potpuno odsustvo specijalizovanih tretmana, nedostatak podrške, kao i osećaj bespomoćnosti staratelja. Vanredni uslovi pandemije otkrivaju nedovoljno razvijene strategije za održavanje podrške ovoj populaciji i potrebu za boljim predviđanjima u budućnosti, kako bi se zaštitili ova ranjiva populacija i njihovi staratelji.

Acta Medica Medianae 2022;61(4):05-11.

Ključne reči: *poremećaji iz spektra autizma, staratelji, podrška, COVID-19*

Original article

UDC: 547.972.2:615.281.9
doi:10.5633/amm.2022.0402

SYNTHESIS AND SCREENING OF ANTIMICROBIAL ACTIVITY OF TWO BROMO-3',4'-DIMETHOXYCHALCONE DERIVATIVES

Valentina Gocić[1], Ana Kolarević[2], Nikola Krstić[1], Jelena Lazarević[3]

Bromochalcone derivatives were synthesized, structurally characterized and screened for their *in vitro* antibacterial activity against a panel of two Gram-positive (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538) and two Gram-negative (*Escherichia coli* ATCC 8739 and *Salmonella typhimurium* ATCC 14028) laboratory control strains in a disc diffusion assay. The antimicrobial test revealed that 4-bromo-3',4'-dimethoxysubstituted chalcone was active against two involved Gram-negative strains, exhibiting stronger bactericidal effect on *E. coli* (11 ± 0.3 mm) than on *S. typhimurium* (15 ± 0.7 mm). Such observed difference in activity highlighted A-ring position 4- as potentially favourable for creating effective Gram-negative antibacterial agents.

Acta Medica Medianae 2022;61(4):12-17.

Key words: *chalcone derivatives, synthesis, antibacterial activity*

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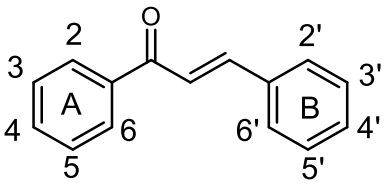


Figure 1. Structural representation of (*E*)-chalcone scaffold

Introduction

Chalcones represent a class of phenolic compounds widely distributed in the kingdom Plantae. Being both intermediates and end products in flavonoid biosynthesis, chalcones act as defensive compounds preventing molecular damage caused by pathogens, pests and herbivores, contributing to the medicinal value of herbs. Structurally this diverse class of plant secondary metabolites can be considered as open-chain flavonoids, representing a precursor unit in flavonoid and isoflavonoid biosynthesis (1). Using chemistry language, chalcones are 1,3-diaryl-2-propen-1-ones in which two benzene rings are joined by an α,β-unsaturated carbonyl system (2) as is shown in Figure 1.

Naturally occurring chalcones and their synthetic derivatives were involved in numerous pharmacological studies, showing a wide range of biological activities. It has been shown that this group of compounds expresses antiarrhythmic, antitrombic, antineoplastic, antiangiogenic, antiinflammatory, antihistaminic, antioxidant, antidiabetic, hypolipidemic, antihypertensive, antimicrobial, antiprotozoal, antiulcer, antigout, immunosuppressant, sedative, hypnotic, antispasmodic, analgesic, estrogenic, vasorelaxant and other diverse activities (3, 4). Taking into account the wide spectrum of their biological activities and the fact that they cannot be isolated from natural sources in large quantities, great efforts have been made towards bioinspired synthesis of chalcone derivatives. Chemical modulations of chalcone scaffold mainly involve the modification of substitution pattern on aromatic rings, but also the replacement of phenyl rings with heteroaryls and formation of hybrid molecules through conjugation with other pharmacologically active compounds (4, 5); this achieves enhanced activity and reduction of toxicity of synthetic analogs in comparison to their

natural counterparts (6). A small number of chalcone derivatives have reached the point of inclusion in clinical studies, some of which are clinically approved for the treatment of several conditions [for example metochalcone used as a choleretic agent, sofalcone as a muciprotective agent and hesperidin methyl chalcone as a vascular protective agent (7)] or registered as ingredients in cosmetic preparations (8, 9).

Chalcones reportedly have exhibited strong inhibitory activities against bacteria that are pathogenic to humans (10). The antibacterial effects are due to reactions between these compounds and the cell membrane of the target microorganism, their ability to attach with outer cell, absorbable proteins and the cell walls (11). Like other biological properties, microbiological activity is generally attributed to the α,β-unsaturated keto moiety and is also found to be dependent on the presence, the number and the position of functional groups such as methoxy, glycosides, hydroxyl, halogens in both phenyl rings (12, 13). Chemical substitutions of the phenyls are also the subject of interest, and useful conclusions about the structure-activity relationship facilitate the synthesis of pharmacologically active chalcones. An excellent review exploring recent developments of chalcones as potential antibacterial agents in medicinal chemistry, summarising the structure-activity relationships and mechanisms of antibacterial action has provided some important guidance for design and synthesis in future (14). Prompted by all these observations, studying the effect of bromine substitution on antimicrobial potential of diverse 1-(bromophenyl)-3-(3′,4′-dimethoxyphenyl) prop-2-en-1-ones, we report herein the synthesis and preliminary antibacterial evaluation of two chalcone derivatives, the A-ring 3- and 4-positional isomers:
(*E*)-1-(3-bromophenyl)-3-(3′,4′-dimethoxyphenyl) prop-2-en-1-one (**4**) and (*E*)-1-(4-bromophenyl)-3-(3′,4′-dimethoxyphenyl) prop-2-en-1-one (**5**).

Experimental

Chemicals

All chemicals used were of analytical reagent grade. Unless specified otherwise, all reagents and standards were purchased from Merck (Darmstadt, Germany).

Chemical synthesis procedure

A procedure for the preparation of (*E*)-1-(3-bromophenyl)-3-(3′,4′-dimethoxyphenyl) prop-2-en-1-one (**4**) and (*E*)-1-(4-bromophenyl)-3-(3′,4′-dimethoxyphenyl) prop-2-en-1-one (**5**).

A mixture of 3- and 4-bromoacetophenones (compounds **1** and **2**, 3 mmol) and 3,4-dimethoxyphenylaldehyde (compound **3**, 3 mmol) was stirred in ethanol (12 mL) and then 60% aqueous solution of sodium hydroxide (3 mL) was added dropwise with continuous stirring at 0 ^{O}C (Scheme 1). The mixture was stirred for 2-3 hours in the ice-bath. The reaction progress was monitored by thin layer chromatography (TLC) on silica gel 60 pre-coated F_{264} plates (Merck), (hexane/ethyl acetate, 4:1). Developed plates were examined with UV lamps (254 nm).

The chalcone derivatives **4** and **5** precipitate out as solids. The mixture was diluted with ice-cold water, filtered under reduced pressure and washed with cold water until neutral pH. The obtained crude products were recrystallized from 96% ethanol. Purity of obtained chalcones was above 95.0% (95.1% for compound **4**, and 99.0% for compound **5**), checked by high-performance liquid chromatography (HPLC), Agilent Technologies 1200 (Wilmington, DE, USA) equipped with photo diode array detector.

NMR analysis

^{1}H and ^{13}C NMR spectra of compounds **4** and **5** were measured at Bruker Avance III – 300 spectrometer. All NMR spectra were recorded at 298 K in $CDCl_3$ (isotopic enrichment 99.95%) solution. Chemical shifts are reported on the δ (ppm) scale and are relative to residual $CHCl_3$ signals (7.24 for ^{1}H and 77.0 ppm, central line, for ^{13}C spectra, respectively) and are given as: s (singlet), d (doublet), t (triplet) or m (multiplet). Scalar coupling constants are reported in hertz (Hz). The experi-mental error in the measured ^{1}H-^{1}H coupling con-stants was ± 0.5 Hz.

Antibacterial activity

Bacterial strains

Antibacterial activity of the synthesized compounds was tested *in vitro* against a panel of laboratory control strains belonging to the American Type Culture Collections, Maryland, USA: two Gram-positive (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538) and two Gram-negative (*Escherichia coli* ATCC 8739 and *Salmonella typhimurium* ATCC 14028) laboratory control strains were obtained from the National Collection of Type Cultures. All microorganisms were maintained at -20 ^{O}C under appropriate conditions and regenerated twice before use in the manipulations.

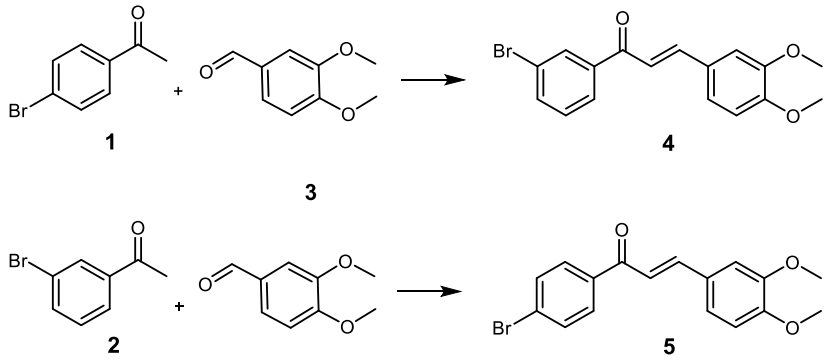
Screening of antibacterial activity

The *in vitro* antibacterial activity of the synthesised compounds **4** and **5** was determined using the disc diffusion assay recommended by NCCLS (15), described in detail in Lazarević et al. (16). For growing the Gram-positive and Gram-negative bacteria, Antibiotic Medium 1 (Difco Laboratories, Detroit, MI, USA) was used. The nutrition medium was prepared according to the instructions of the manufacturers. All agar plates were prepared in 90 mm Petri dishes with 22 mL of agar, giving the final depth of 4 mm. A suspension of the tested microorganisms (0.1 mL, 10^{8} cells per mL) was spread on the solid media plates. Dimethyl sulph-oxide (10%

aqueous solution) was used to dissolve and to dilute samples to the highest concentration to be tested (stock concentrations 1 mg/mL). Sterile filter paper disks ("Antibiotica Test Blättchen", Macherey-Nagel, Düren, Germany, 9 mm in diameter) were impregnated with 10 µL of the tested sample solution and placed on the inoculated plates. These plates, after standing at 4 ^{O}C for 2 h, were incubated at 37 ^{O}C for 24 h. Standard disks of tetracycline and gentamicin (Institute of Immunology and Virology "Torlak", 30 µg of the active component, diameter 6 mm) were used as the positive controls, while disks imbued with 10 µL of 10% dimethyl sulphoxide were used as the negative controls. The diameters of the inhibition zones were measured in millimetres using a "Fisher-Lilly Antibiotic Zone Reader" (Fisher Scientific Co., USA). Each experiment was replicated three times. Mean values are presented.

Results and discussion

Chemistry and spectral data on synthetized compounds: Reaction of 3- (**1**) and 4-bromoacetophenone (**2**) with 3,4-dimethoxybenzaldehyde (**3**) in basic medium, formed chalcone derivatives **4** and **5** (Scheme 1) which were characterized using ^{1}H and ^{13}CNMR experiments (Supplemental data). The reaction led to the expected products in high yield and in pure form (purity confirmed by HPLC).



Scheme 1. Synthesis of chalcone derivatives **4** and **5**

(*E*)-1-(3-Bromophenyl)-3-(3',4'-dimethoxyphenyl) prop-2-en-1-one (4)

Yield 79.7%; ^{1}HNMR (300 MHz, $CDCl_3$) δ (ppm): 8.13 (s, ^{1}H), 7.93 (d, J = 7.6 Hz, ^{1}H), 7.77 (d, J = 15.6 Hz, ^{1}H), 7.71–7.69 (m, ^{1}H), 7.38 (t, J = 8.0 Hz, ^{1}H), 7.32 (d, J = 15.6 Hz, ^{1}H), 7.26–7.23 (m, ^{1}H), 7.16 (s, ^{1}H), 6.91 (d, J = 8.0 Hz, ^{1}H), 3.95 (d, J = 9.2 Hz, ^{6}H).

^{13}CNMR (300 MHz, $CDCl_3$), δ (ppm): 187.7, 151.5, 149.0, 145.4, 139.9, 135.5, 130.9, 130.8, 127.5, 127.3, 124.3, 122.3, 119.1, 111.5, 111.1, 55.8.

(*E*)-1-(4-bromophenyl)-3-(3',4'-dimethoxyphenyl) prop-2-en-1-one (5)

Yield 89.3%; ^{1}HNMR (300 MHz, $CDCl_3$): δ (ppm): 7.88 (d, J = 8.4 Hz, ^{2}H), 7.77 (d, J = 15.6 Hz, ^{1}H), 7.64 (d, J = 8.4 Hz, ^{2}H), 7.33 (d, J = 15.6 Hz, ^{1}H), 7.25–7.23 (m, ^{1}H), 7.16 (s, ^{1}H), 6.91 (d, J = 8.4 Hz, ^{1}H), 3.95 (d, J = 6.8 Hz, ^{6}H).

^{13}CNMR (300 MHz, $CDCl_3$), δ (ppm): 188.4, 151.4, 149.0, 145.1, 136.8, 2x131.7, 2x130.4, 127.4, 127.0, 124.2, 119.2, 111.5, 110.8, 55.7, 55.6.

In vitro antibacterial activities. The antimicrobial activity was evaluated in a disc diffusion method, measuring inhibition zones of bacterial growth. The results of preliminary antibacterial testing are presented in Table 1 from which can be seen that not all of the tested compounds were equally effective against the selected bacterial strains. While compound **5** was active, exhibiting stronger bactericidal activity on *E. coli* (11 ± 0.3 mm) than on *S. typhimurium* (15 ± 0.7 mm), a complete absence of activity was observed for compound **4**. Effecting the growth of only Gram-negative strains, compound **5** exhibited also selectivity. The assayed samples were less effective than antibiotics used as reference standards.

To the best of our knowledge, our work is the first study that tested **4** and **5** on *B. subtilis*, *S. aureus*, *E. coli* and *S. typhimurium*. Compound **5** was previously involved in only one antimicrobial study, evaluating growth of avirulent and virulent mycobacteria, however no marked effect on inhibition of mycobacterial growth was observed (17).

Results for the tested compounds from our (antibacterial) activity study together with large number of published papers (14, 18-20) clearly

show that the structure-activity relationship for the A-ring substituted chalcones is strongly conditioned by the position of the halogen. Seems that A-ring bromo-substitution in position 4- for 3′,4′-dimethoxysubstituted chalcones is more favourable for creating active Gram-negative antibacterial agents than the position 3- (Table 1, compounds **4** and **5**).

Table 1. The antimicrobial activity (diameters of growth inhibition zones measured in mm) of 3- and 4-bromo-3′,4′-dimethoxysubstituted chalcones (compounds **4** and **5**, respectively) and of positive (antibiotics tetracycline and gentamicine)/negative (DMSO 10% aqueous solution) control.

	Microorganisms			
	Gram-positive		Gram-negative	
Cpd. entry	*B. subtilis*[a]	*S. aureus*	*E. coli*	*S. typhimurium*
4	na[b]	na	na	na
5	na	na	11 ± 0.3	15 ± 0.7
Tetracycline[c]	32.1 ± 0.5	30.7 ± 0.5	30.5 ± 0.5	31.2 ± 0.7
Gentamicine	22.1 ± 0.3	19.2 ± 0.7	24.2 ± 0.9	22.2 ± 0.3
DMSO 10% aqueous solution[d]	na	na	na	na

[a]Mean value ± SD (in mm) of five experiments, including disc diameter, 9 mm (10 µl per disc).
Values representing bactericidal zones in which the growth of bacteria was not observed.
[b]Not active.
[c]Positive control bactericidal activity (30 µg per disc).
[d]Negative control (10 µl per disc)

Conclusion

In this paper, we have described synthesis and antibacterial evaluation of two chalcone derivatives. To synthesize the compounds, eco-friendly and easy method has been used, including mild reaction conditions, use of recyclable solvent and easy work-up procedures. The products were obtained in high yield and in pure form. Compounds were evaluated for their *in vitro* antimicrobial activities in disc diffusion assay. Based on the results of two synthetized and tested samples as well as on the basis of numerous papers published, most likely the structure-activity relationship for 3- and 4-bromo-substituted chalcones is strongly conditioned by the position of the halogen. For 3- and 4-bromo 3′,4′-dimethoxysubstituted chalcones a difference in activity highlights position 4- as potentially favourable for creating effective Gram-negative antibacterial agents.

Acknowledgement

The work was funded by the Ministry of Science and Technological Development of Serbia (Project 451-03-68/2022-14/200113) and Faculty of Medicine, University of Niš Internal project No. 40.

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Originalni rad **UDC: 547.972.2:615.281.9**
doi:10.5633/amm.2022.0402

SINTEZA I PROCENA ANTIMIKROBNE AKTIVNOSTI MONOSUPSTITUISANIH DERIVATA 3',4'-DIMETOKSIHALKONA

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Monosupstituisani 3',4'-dimetoksihalkonski derivati sintetisani su bazno katalizovanom Klajzen-Šmitovom kondenzacijom, strukturno okarakterisani i podvrgnuti *in vitro* ispitivanju antibakterijske aktivnosti na laboratorijske sojeve Gram-pozitivnih (*Bacillus subtilis* ATCC 6633 i *Staphylococcus aureus* ATCC 6538) i Gram-negativnih (*Escherichia coli* ATCC 8739 i *Salmonela typhimurium* ATCC 14028) bakterija. 4-Brom-3',4'-dimetoksihalkon deluje baktericidno na Gram-negativne sojeve, ispoljavajući snažniji antimikrobni efekat prema *E. coli* (11 mm ± 0,3 mm), u odnosu na *S. typhimurium* (15 mm ± 0,7 mm). Rezultati antimikrobnog testa ukazuju na potencijalni značaj pozicije 4-A-prstena halkona u kreiranju antibakterijskih agenasa selektivnog dejstva.

Acta Medica Medianae 2022;61(4):12-17.

Ključne reči: *halkonski derivati, sinteza, antibakterijska aktivnost*

Original article

UDC: 616.895.8:577.15:575.113.2
doi:10.5633/amm.2022.0403

ANALYSING CYP2D6*4 ALLELE FREQUENCY IN PATIENTS WITH SCHIZOPHRENIA

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Cytochrome P450 enzyme superfamily is involved in the metabolism of a range of endogenous and exogenous substrates. The CYP2D6 variant is involved in the metabolism of dozens of drugs such as tricyclic antidepressants, antipsychotics, beta-blockers, anti-arrhythmics, anti-diabetics, anticancer drugs and so on. CYP2D6 enzyme exhibits high polymorphism and the most frequent variant allele CYP2D6*4 is a poor metabolizer (PM). PM causes the reduction of therapeutic response, increase the risk of adverse drug reactions and increase the plasma concentration of both drug and its metabolites above the levels of toxicity. The aim of this study was to analyze CYP2D6*4 allele frequency among schizophrenic patients for further individualisation and rationalisation of therapy. For that purpose we recruited 38 schizophrenic patients and 110 healthy individuals. Allele-specific PCR was used to detect of CYP2D6*4 allele. In 55% of schizophrenic patients we found both wild type allele carriers, in 45% wild type/*4 heterozygous, while *4/*4 homozygous was not identified. A statistically significant difference in the genotype distribution ($p < 0.05$) between schizophrenic patients and healthy individuals was noted. The frequency of allele *4 (37%) is significantly higher in schizophrenics compared to controls, which indicates caution in administration of CYP2D6 substrates. A lower frequency of PMs in schizophrenic patients than in healthy individuals could be explained with CYP2D6 neuroactive substrate metabolism. However, 45% of the schizophrenic patients, who are intermediate metabolizers, carry the higher risk of adverse response to CYP2D6 substrates comparing to wild type. Since none of the analyzed patient was PM, it can be concluded that they received an adequate dose of medication.

Acta Medica Medianae 2022;61(4):18-23.

Key words: *schizophrenia, CYP2D6*4, allele, allele specific PCR*

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Introduction

Cytochrome P450 2D6 (CYP2D6) is a member of the cytochrome P450 mixed-function oxidase system. This family is involved in various oxidation/ reduction reactions used in the metabolism of a range of endogenous and exogenous substrates (1). Although the 2D6 isoenzyme is represented by 1.5%, it metabolizes 25% of drugs that are metabolized via cytochrome, which makes up 7-10% of all drugs on the market (2). The CYP2D6 enzyme variant exhibits high polymorphism due to allelic combinations more than 100 different alleles. Most people carry two functional alleles (CYP2D6*1- "wild type" or CYP2D6*2) and are able to metabolize CYP2D6 substrates extensively. In 7-10% of Caucasian Europeans, CYP2D6 enzyme activity is absent due to inheritance of two non-functional alleles (e.g. CYP2D6*3, *4, *5, *6). These individuals are classified as slow (poor) metabolizers (PM). People with one non-functional and one functional allele are considered intermediate metabolizers (IM). The term is also used for persons with one non-functional allele and one allele encoding an enzyme with reduced function, or for persons in whom both alleles encode an enzyme with reduced function (e.g. *10, *17, *41). Ultra-fast metabolizers (UM) have more than two functional copies of the 2D6 gene (mainly CYP2D6*2), exhibit very intense enzymatic activity, are most common among residents of Northeast Africa and Saudi Arabia, while the incidence in Europeans is 1-5% (3-6). Generally, it has been shown that mutations of CYP2D6 are more prevalent in certain countries in the African and Asian continents than in some European countries (2, 7). In a South India, the prevalence of

CYP2D6 alleles in the descending order was CYP2D6*1, *2, *10, *4, and *5 (8). CYP2D6*4 is the most frequent variant allele in Caucasian Europeans (20%) and in the Middle Eastern countries (9), and it is the leading cause of the slow metabolizer phenotype. Over 75% of slow metabolizers are carriers of this polymorphism (8). The most common inactivating mutation in Caucasian Europeans is the G1934A substitution at the intron extrusion site (CYP2D6*4 allele), which leads to the synthesis of truncated protein and the absence of catalytic activity. G u A transition at the intron 3/exon 4 boundary of the CYP2D6 gene causes improper excretion of introns on the mRNA and results in mutation of the transcription frame shift and premature termination. The G to A transition has been identified as a primary defect of the CYP2D6 locus and is present in 80-90% of mutant alleles in slow metabolizers (10).

Due to the decrease in CYP2D6 activity, the metabolism of drugs, that are primarily metabolized via CYP2D6 decreases, which increases the plasma concentrations of these drugs and increases the risk of dose-dependent side effects. Fast metabolizers (*1/*1 homozygotes) are thought to carry a lower risk of developing drug intolerance, dose-dependent adverse reactions and drug toxicity symptoms compared to intermediate metabolizers (1*/4* heterozygotes). Slow metabolizers require lower maintenance doses compared to fast metabolizers. There are insufficient data on initial doses, time to target plasma drug concentrations, first dose effects, and response to change in therapy, most likely due to the large number of different pharmacological groups of drugs metabolized by CYP2D6 (6, 8).

Schizophrenia is the most devastating chronic psychiatric disorder expressing in many different clinical forms. Clinically, patients may show positive symptoms (delusions, hallucinations, agitation or catatonia), negative symptoms (social withdrawal with lack of affective responses, apathy, anhedonia and impaired thought and speech content) or may exhibit both types of symptoms simultaneously. For the treatment of such complex symptomatology, doctors have at their disposal a range of antipsychotic drugs that are equally effective in the treatment of psychotic symptoms and differ in the type of their side effects and sedative effect. First-generation antipsychotics (chlorpromazine) cause a number of side effects of anticholinergic and extrapyramidal origin. Therefore, the first line in the treatment of schizophrenia consists of second-generation antipsychotics (olanzapine, risperidone and quetiapine), which cause fewer side effects. Clozapine is reserved for the treatment of resistant schizophrenia and requires careful monitoring and control, because in addition to the characteristic side effects, it also causes severe agranulocytosis (11). The concentration of antipsychotics in plasma is particularly affected by the metabolism of CYP2D6, which is of great importance due to the narrow therapeutic index of these drugs. Side effects (perphenazine, haloperidol and thioridazine) such as excessive sedation and Parkinson's side effects are associated with changes in CYP2D6 metabolism, while the effect on tardive dyskinesia, acute dystonia of extrapyramidal symptoms and akathisia has not been established (7, 8, 12). As the 2D6 isoenzyme is the only non-inducible among CYP450 enzymes and is not regulated by any known environmental factors, genetic variations contribute greatly to inter-individual differences in enzyme activity (8).

Materials and methods

Patients

A total of 148 subjects, 38 patients with schizophrenia and 110 healthy subjects were included in the study. Patients were recruited in the Specialized Hospital for Psychiatric Diseases "Gornja Toponica" in Niš, with a diagnosis of chronic schizophrenia, who were on appropriate pharmacological therapy. Psychiatric symptoms were monitored using The Positive and Negative Syndrome Scale (PANSS).

CYP2D6*4 genotyping

Genetic testing of CYP2D6 polymorphism was performed in the Laboratory for Functional Genomics and Proteomics of the Scientific Research Center for Biomedicine of the Medical Faculty in Niš. DNA was isolated from whole blood samples supplemented with EDTA, standard Na-dodecyl sulfate lysis procedures, proteinase K digestion, phenol/chloroform extraction and ethanol precipitation, and a commercial isolation kit (Fermentas, Terhmo Fischer Scientific Inc). Detection of CYP2D6*4 was performed by amplification of the desired gene segment, allele specific polymerase chain reaction (ASPCR), using 4 primers, marked as:

1: 5'-TCCCAGCTGGAATCCGGTGTCG-3'
2: 5'-GGAGCTCGCCCTGCAGAGACTCCT-3'
7: 5'-CGAAAGGGGCGTCC-3'
11: 5'-TCTCCCACCCCCAA-3'

The 25 μL PCR reaction mixture contained: 12.5 μL Kappa Mix (Fermentas), 10.2 μL ultrapure water, 0.5 μL primer 1, 0.5 μL primer 2, 0.5 μL primer 7, 0.5 μL primer 11 and 0.3 μL DNA (50 ng/mL). The amplification program of the CYP2D6 gene allele 4 is shown in Table 1.

The obtained PCR products were further analyzed by horizontal electrophoresis, on a 1.5% agarose gel with TBE buffer and ethidium bromide as amplifier detection agent, for 1.25 h, and detection was performed on a transilluminator under a UV lamp. DNA was subsequently reanalyzed to confirm the results of the CYP2D6*4 assay.

Statistical analysis. Statistical processing of the results was performed using the SPSS statistical program 15.0, χ^2 test and Fisher's accuracy test. $P < 0.05$ was considered statistically significant.

Results

By allele-specific PCR, CYP2D6*4 allele was detected as slow metabolizing form of CYP enzyme. A 750 bp fragment was amplified first (primers 1 and 2), followed by ASA PCR to give 560 bp(wt) and 217 bp(*4) fragments. The distribution of genotypes in patients with schizophrenia and non-schizophrenics is shown in Table 2.

The distribution of genotypes in patients with schizophrenia is significantly different compared to healthy subjects. A heterozygote wild-type/*4 was identified in 45% of patients with schizophrenia, as opposed to 22% in controls, while slow metabolizing homozygotes were not observed and significantly lower ($p < 0.05$) compared to the control group. The incidence of CYP2D6*4 alleles was significantly higher in patients with schizophrenia (Table 3) compared to the reference group of control subjects ($p < 0.0001$).

Table 1. CYP2D6 amplification program

Program			
Order of			
1.		Temperature	Time
		95 °C	2 minutes
2.	**First set**		
	Number of cycles	12	
		Temperature	Time
		95 °C	15 seconds
		63 °C	30 seconds
		72 °C	45 seconds
3.	**Second set**		
	Number of cycles	24	
		Temperature	Time
		95 °C	15 seconds
		53 °C	30 seconds
		72 °C	45 seconds
4.	**Final elongation**		
		Temperature	Time
		72 °C	7 minutes

Table 2. Genotype distribution in the CYP2D6 gene in patients with schizophrenia

Genotype	Patients with schizophrenia	Control group
wt/wt	55%	75%
wt/*4	45% ***	22%
*4/*4	-	3%*

* $p < 0.05$; *** $p < 0.001$

Table 3. Frequency of CYP2D6*w or 4 alleles in patients with schizophrenia

Allele	Patients with schizophrenia	Control group
Wild-type	63%	86%
CYP2D6*4	37% ***	14%

*** $p < 0.0001$

Discussion

In this study, a significant difference in genotype distribution was noted, as well as a significantly higher frequency of alleles of *4 of CYP2D6 gene in patients with schizophrenia compared to healthy individuals. There is also a significantly lower prevalence of slow metabolizing homozygotes (*4/*4) in schizophrenic patients than in healthy volunteers. This finding is consistent with the results of other similar studies and could be explained by the involvement of CYP2D6 in the metabolism of endogenous neuroactive substrates (13, 14). It also supports the hypothesis of a potential role of CYP2D6 in the vulnerability to schizophrenia. In the previously published study (15), a significant clinical improvement was found in patients with schizophrenia with the CYP2D6 poor metabolizer phenotype compared with the treatment out comes in extensive metabolizers. However, Kakihara et al. (16) did not identify any significant association between CYP2D6 polymorphisms and clinical recovery. This finding was supported with another study involving female patients with schizophrenia that found clinical improvement following risperidone treatment, but was not associated with CYP2D6 genotype (17). Recently, Lu et al. (18), in a well-designed study on the effect of CYP2D6 polymorphism on the concentration and therapeutic effects of risperidone, have shown that the CYP2D6 genotype exerts a slight effect on improvement of clinical symptoms but has a significant effect on risperidone plasma concentrations. This result suggests that in patients with schizophrenia treated with risperidone CYP2D6 genotype might influence adverse drug reactions. There is evidence that children with CYP2D6 poor or intermediate metabolizer phenotypes are at a greater risk for risperidone adverse effect (19). It was also shown that the plasma concentration of risperidone was significantly different depending on homo - or hetrozygosity of CYP2D6*10 mutations confirming the finding of previous studies showing that homozygous mutations CYP2D6*10 had higher plasma concentrations of risperidone than single-allele carriers (20, 21). Lu et al. (18) also showed that C100T and G4181C polymorphisms were associated with differences in plasma concentration of risperidone and the ratio of risperidone to 9-hydroxyrisperidone suggesting that even single-nucleotide mutations are sufficient to affect the activity of enzymes. It is obvious that plasma concentration varies according to allelic variants. Importantly, this may contribute to the clinical treatment response and may provide new insight for individualized drug treatment.

In contrast to the results obtained on the frequency of slow metabolizers, we found a significantly higher prevalence of CYP2D6*4 alleles and intermediate metabolizers in patients with schizophrenia which is consistent with the findings of Llerena et al. (13). Very recently (22), it has been shown that physiologically based pharmacokinetic model for aripiprazole and dehydro-aripiprazole indicates a dose reduction for CYP2D6 poor metabolizers to achieve steady-state plasma concentrations and suggests a maximum daily dose of 10 mg for patients with schizophrenia, while IMs and UMs do not need a dose adjustment. Further, dose adjustment to the CYP2D6 genotype or phenotype according to the guidelines is not applicable for patients already using antipsychotics (23). That the polymorphism of CYP2D6*10 affects the steady state plasma concentration of risperidone is also confirmed in Indian patients with schizophrenia (24). By examining the possible association between CYP2D6 genotype, hippocampal white matter integrity, and therapeutic responseto antipsychotic drugs in Korean patients with schizophrenia Shin et al. (25) showed that CYP2D6-dependent hippocampal white matter alterations could be an endotype for schizophrenia that accounts for individual differences in clinical features and treatment responses.

Conclusion

Taken together, our results, although we did not identify poor metabolizers, suggest CYP2D6 genotyping combined with drug concentration monitoring in order to personalize the drug dose to achieve efficacy and avoid adverse effects in patients with schizophrenia.

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Originalni rad **UDC: 616.895.8:577.15:575.113.2**
doi:10.5633/amm.2022.0403

ANALIZA FREKVENCIJE ALELA CYP2D6*4 KOD BOLESNIKA SA SHIZOFRENIJOM

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Superfamilija enzima citohroma P450 uključena je u metabolizam niza endogenih i egzogenih supstrata. Varijanta CYP2D6 uključena je u metabolizam desetina lekova, kao što su triciklični antidepresivi, antipsihotici, beta blokatori, antiaritmici, antidijabetici, lekovi protiv karcinoma i tako dalje. Enzim CYP2D6 pokazuje visok polimorfizam i najčešća varijanta alela CYP2D6*4 je spor metabolizator (SM). SM uzrokuje smanjenje terapijskog odgovora, povećava rizik od neželjenih reakcija na lek i povećava koncentraciju leka i njegovih metabolita u plazmi, iznad nivoa toksičnosti. Cilj ove studije bila je analiza učestalosti alela CIP2D6*4 kod bolesnika sa shizofrenijom, radi dalje individualizacije i racionalizacije terapije. U ispitivanje je uključeno 38 bolesnika sa shizofrenijom i 110 zdravih osoba. PCR specifičan za alel korišćen je za detekciju alela CYP2D6*4. Kod 55% bolesnika sa shizofrenijom pronašli smo oba nosioca alela divljeg tipa, kod 45% heterozigot divlji tip/*4, dok homozigot *4/*4 nije identifikovan. Uočena je statistički značajna razlika u distribuciji genotipa ($p < 0,05$) između shizofrenih bolesnika i zdravih osoba. Učestalost alela *4 (37%) značajno je veća kod obolelih od shzofrenije u poređenju sa ispitanicima kontrolne grupe, što ukazuje na oprez u primeni supstrata CYP2D6. Niža učestalost SM kod shizofrenih bolesnika, nego kod zdravih osoba može se objasniti metabolizmom neuroaktivnog supstrata CYP2D6. Međutim, 45% bolesnika sa shizofrenijom, koji su srednji metabolizatori, nosi veći rizik od neželjenog odgovora na supstrate CYP2D6, u poređenju sa divljim tipom. Kako nijedan od analiziranih bolesnika nije bio SM, može se zaključiti da su bolesnici primali adekvatne doze leka.

Acta Medica Medianae 2022;61(4):18-23.

Ključne reči: *shizofrenija, CYP2D6*4, alel, alel specifična PCR*

Original article

UDC: 615.015.2:616.72-002.77
doi:10.5633/amm.2022.0404

INFLUENCE OF POLYPHARMACY ON THE FUNCTIONAL ABILITY OF PATIENTS WITH RHEUMATOID ARTHRITIS

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Clinical guidelines for the treatment of rheumatoid arthritis (RA) are based on the use of: nonsteroidal anti-inflammatory drugs (NSAIDs), conventional disease-modifying drugs (csDMARDs), immunosuppressants and biological therapies. Unfortunately, a large number of patients have other chronic diseases in addition to RA, which requires additional multiple use of drugs and often leads to polypharmacy. According to the definition of the World Health Organization (WHO), polypharmacy is defined as the routine use of 5 or more drugs, including prescription of over-the-counter drugs, dietary supplements, and traditional medicines. The aim of the study was to examine the impact of polypharmacy on the functional ability of patients with RA estimated based on the HAQ-DI index.

A retrospective study included 131 patients diagnosed with RA. Within the clinical characteristics, attention was focused on the present comorbidities, therapy and the total number of drugs used. The HAQ questionnaire was used to assess health status and functional ability. The obtained data were analyzed and statistically processed using appropriate software and statistical methods.

The study involved 29 males and 102 females mean age 60.25 ± 11.21 years. The analysis of the collected clinical data showed the presence of comorbidities in 80.15% of patients. Synthetic disease-modifying drugs were used 88.55%, while 13.74% of patients were on biological therapy. HAQ-DI values < 1.5 were present in 83.21%, while HAQ-DI ≥ 1.5 values were recorded in 16.79% of respondents. Polypharmacy was present in 75.57% of respondents. Potential interactions were more frequent in the group of respondents who can perform normal physical activities without or with mild restrictions (HAQ-DI < 1.5), while the presence of one serious potential interaction was more pronounced in the group of respondents with reduced functional ability (HAQ-DI ≥ 1.5).

The results of this study show a high frequency of polypharmacy and consequent potential drug interactions in patients with RA. Accordingly, monitoring of polypharmacy in patients with RA is necessary in order to achieve optimal functional status, disease control, minimize drug interactions and side effects.

Acta Medica Medianae 2022;61(4):24-30.

Key words: *Polypharmacy, Rheumatoid arthritis, HAQ-DI*

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Introduction

The incidence of rheumatoid arthritis (RA) is estimated to be 0.5-1% worldwide, while the majority of patients are able-bodied patients (1). The disease is manifested by joint pain, fatigue, worsening of the emotional state, and if left untreated, there is irreversible structural and functional damage and functional incapacity of patients (2).

With the development and improvement of pharmacotherapy, the success in treatment is much greater compared to the epochs when there were no known drugs that modify the course of the disease (3). The goal of RA treatment is not only based on remission of the disease, but also on minimizing the consequences of the disease in order to increase the functional ability and quality of life of patients (4, 5). In this regard, quality of life is used as a subjective unit of measurement of the condition of patients suffering from this disease (6). The Health Assessment Questionnaire (HAQ) is the most commonly used questionnaire to assess the functional ability of patients with RA. Factors contributing to functional disability include: age, female gender, disease acti-

vity, rheumatoid factor (RF) or anti-citrulline antibodies (AntiCCP), radiological impairment, number of comorbidities present, and socioeconomic status (7-11).

Clinical guidelines for the treatment of RA are based on the use of nonsteroidal anti-inflammatory drugs (NSAIDs), conventional disease-modifying drugs (csDMARDs), immunosuppressants and biological therapies. The use of the mentioned drugs achieves optimal pharmacotherapeutic goals in the treatment of RA. However, patients with RA are significantly burdened by other diseases compared to the general population, which carries a risk of polypharmacy (12, 13). According to the definition of the World Health Organization (WHO), polypharmacy is defined as the routine use of 5 or more drugs, and this including prescription or over-the-counter drugs, as well as dietary supplements and traditional medicines (14). It may be useful to patients if the patient's clinical conditions, comorbidities, allergies, potential drug-drug interactions, drug-disease interactions are taken into account, and if drug prescribing is based on clinical evidence (15). Today, polypharmacy is a major public health challenge, primarily due to the increased likelihood of experiencing side effects that may affect a patient's health (16, 17).

The aim of the study was to examine the impact of polypharmacy on the functional ability of patients with RA estimated based on the value of the HAQ-DI index.

Patients and methods

The research was conducted at the Clinic for Rheumatology of the Military Medical Academy in Belgrade. The conducted retrospective research included 131 patients diagnosed with rheumatoid arthritis, treated in the period between 2019 and 2020 by doctors from the Clinic for Rheumatology of the Military Medical Academy. Information on the demographic and clinical characteristics of patients was collected on the basis of medical documentation. As part of the clinical characteristics, attention was focused on the present comorbidities, therapy and the total number of drugs used, while the BNF (British National Formulary) database was used to determine the persistence of potential interactions between prescribed drugs.

The HAQ questionnaire was used to assess the health status and functional status of patients. The questionnaire consists of eight categories of questions that go through the assessment of patients' difficulty in performing normal activities during the day in the previous week. Categories include assessment in performing the following activities: dressing, grooming, getting up, taking food, walking, performing personal hygiene, reaching out, catching, and performing other common activities. Based on the values of the HAQ-DI index, the respondents were divided into two groups (HAQ-DI < 1.5 and HAQ-DI ≥ 1.5). HAQ-DI values < 1.5 indicate that patients can perform normal physical activities without or with mild limitations, while values ≥ 1.5 indicate significant limitations in performing daily activities that can lead to complete inability to perform the same.

Results

The study involved 131 patients, 29 males and 102 females with an average age of 60.25 ± 11.21 years. Demographic and clinical characteristics of the respondents are shown in Table 1.

Table 1. Demographic and clinical characteristics of respondents

Gender (men/women)	29/102 (22.14%/77.86%)
Age	60.25 ± 11.21
Presence of comorbidities (yes/no)	105/26 (80.15%/19.85%)
Synthetic DMARD Biological DMARD	116 (88.55%) 18 (13.74%)
HAQ-DI	< 1.5: 109 (83.21%) ≥ 1.5: 22 (16.79%)
Presence of polypharmacy	99 (75.57%)
Presence of polypharmacy in relation to HAQ-DI values	< 1.5: 83 (76.15%) ≥ 1.5: 16 (72.73%)
Presence of interactions (yes/no)	86/45 (65.65%/34.35%)
Presence of interactions with respect to HAQ-DI	< 1.5: 69 (80.23%) ≥ 1.5: 17 (19.77%)

The analysis of the collected clinical data showed the presence of comorbidities in 105 (80.15%) patients, and the most common were hypertension (44.27%) and osteoporosis (26.72%). Synthetic disease-modifying drugs were used 88.55%, while 13.74% of patients were on biological therapy. In the further course of the research, the functional status of the respondents was analyzed. HAQ-DI values < 1.5 were present in 109 (83.21%), while HAQ-DI values ≥ 1.5 were present in 22 (16.79%) respondents.

Polypharmacy was present in 75.57% of respondents, while a total of 164 potential interactions were identified, of which 16 (9.76%) were mild, 27 (16.46%) moderate and 121 (73.78%) severe. Polypharmacy and drug interactions were more pronounced in the group of respondents who could perform normal physical activities without or with mild restrictions (HAQ-DI < 1.5).

Table 2 shows logistic univariate and multivariate regression when the HAQ-DI value is considered as a dependent variable.

Since a larger number of drugs in therapy is closely related to a larger number of interactions, only one parameter remained significant in the regression analysis.

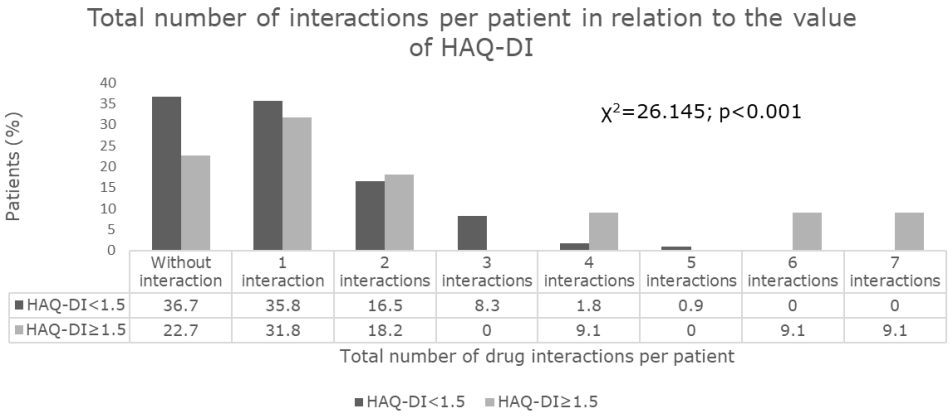
Graph 1 shows the total number of interactions per patient in relation to the HAQ-DI value.

Table 2. Logistic univariate and multivariate regression when HAQ-DI ≥ 1.5 is observed as a dependent variable

Univariate linear regression					
Independent variable	B	95% CI for B	Beta	R^2 (%)	SIG
Gender (female)	-0.042	-0.246-0.162	-0.036	0.1	0.684
Age	0.009	0.002-0.017	0.211	4.4	0.016*
Total number of comorbidities	0.077	0.007-0.148	0.188	3.5	**0.032**
Total number of drugs	0.052	0.024-0.081	0.302	9.1	**< 0.001***
Total number of RA drugs	0.026	-0.037-0.088	0.072	0.5	**0.414**
Total number of interactions	0.115	0.040-0.191	0.314	9.8	**0.003**
DAS28*	0.174	0.130-0.218	0.569	32.3	**< 0.001**

Multivariate linear regression						
Independent variable	B	95% CI for B	Beta	SIG par	R^2 (%)	SIG mod
Age	0.004	-0.002-0.011	0.089	0.210	**40.6**	**< 0.001**
Total number of comorbidities	0.030	-0.039-0.098	0.072	0.390		
Total number of drugs in therapy	0.016	-0.015-0.047	0.094	0.299		
Total number of interactions	**0.056**	**0.001-0.112**	**0.166**	**0.046**		
DAS28*	**0.157**	**0.114-0.200**	**0.514**	**< 0.001**		

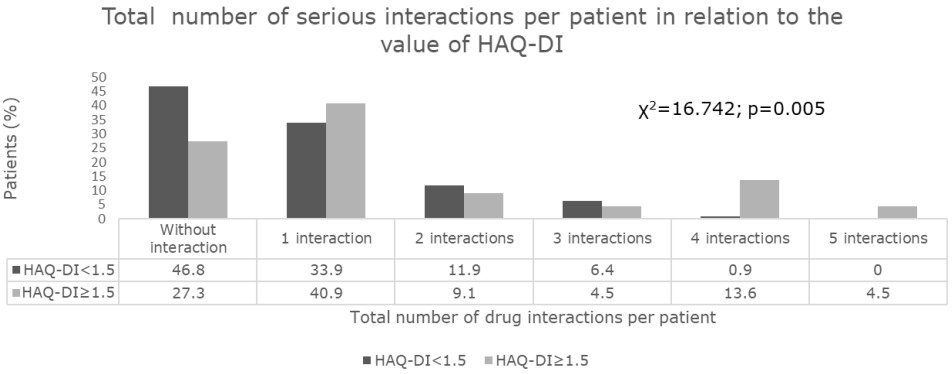
* DAS28 – Disease Activity Score in 28 Joints



Graph 1. Total number of potential interactions per patient in relation to the HAQ-DI value

The analysis of the obtained results showed that the most frequent presence of one interaction (35.8% of patients whose HAQ-DI value was < 1.5 and 31.8% in patients with HAQ-DI ≥ 1.5). A maximum of seven potential interactions (9.1%) were recorded in the group of patients whose HAQ-DI value was ≥ 1.5. Among the examined groups, statistical significance was noted (χ^2 = 26.145; p < 0.001).

The total number of serious interactions per patient in relation to the HAQ-DI value is shown in Graph 2.



Graph 2. Total number of potential serious interactions per patient in relation to the HAQ-DI value

Based on the obtained results, it is found that the presence of one serious interaction is more common (40.9%) in patients with HAQ-DI ≥ 1.5. The highest number of serious interactions was 5, with 4.5% of patients present. Among the examined groups, statistical significance was noted (χ^2 = 16.742; p = 0.005).

Discussion

The course of RA can be mild with spontaneous remissions, which makes it undiagnosed for a long time, but also extremely fast and exhausting for the patient. However, most patients have a moderate form of RA with exacerbation episodes present (18). Early initiation of treatment with regular monitoring of disease activity allows clinicians to adequately identify the most appropriate therapeutic treatment options. This is extremely important to prevent disease progression. By conducting strict control of the disease, doctors can prevent the radiographic development of the disease, improve the performance of physical acti-vities, which ensures a better quality of life for patients (19). By applying the HAQ-DI questionnaire, doctors gain insight into the patient's subjective condition, before starting any therapy, and later it can be used to assess the effectiveness of the therapeutic response, which simplifies the decision on further treatment modalities.

The results of the conducted research show that polypharmacy was present in 75.57% of patients. Polypharmacy can significantly interfere with the treatment and outcome of the disease. The most common consequences of polypharmacy are side effects and interactions, which lead to increased treatment costs and, in the worst case, death (20). A high rate of polypharmacy within RA patients has been reported in studies conducted in Brazil and the United Kingdom. In a study conducted by Gomides AP et al. (20) in a group of 792 patients with RA, the frequency of polypharmacy was shown depending on the HAQ-DI value, with values in the range of 0-1 being represented in 60.16% of patients, while for values greater than 1 it was present in 78.36% of patients from that subgroup. They also showed that there was an association in a subgroup of patients with HAQ-DI values greater than 1 with polypharmacy (20). The results of the mentioned study are in accordance with the results of the conducted research.

A similar study was conducted by Filkova M et al. (21) in which she showed that the presence of polypharmacy greatly affected HAQ-DI values, with functional ability being reduced most often in patients who had more than 5 drugs in their therapy. A couple of years earlier, the same team dealt with a similar issue and showed that HAQ-DI values increased with the increase in the number of drugs used to treat underlying and other present diseases in patients (22). A study conducted by Treharne GJ et al. (23) also showed a significant correlation of polypharmacy with HAQ-DI values, not only with it but also with age, disease duration and DAS28 values, which is consistent with the results of the study.

Bechman K et al (24) conducted a large study whose results indicated that HAQ-DI values were higher in patients who had 6-9 and more than 9 drugs in the treatment of primary and other chronic

and acute diseases. They have shown that polypharmacy has a great impact on reducing the functional abilities of patients suffering from rheumatoid arthritis (24). As a main conclusion, they stated that any new introduction of the drug with the already existing therapy with modifying drugs could lead to a decrease in the effectiveness of the therapeutic response by 8% at the beginning of biologic therapy, while increasing by 13% the chance of developing serious adverse events (24).

Drug-related problems (DRPs) are events or circumstances related to therapy that may or may not interfere with desired health outcomes, and drug-drug interactions can be classified as DRPs. There is a high incidence of DRP in patients with RA, while the incidence is higher in patients with more comorbidities due to polypharmacy and complex treatment regimens (25). In a study conducted in 65.65% of respondents, possible drug interactions were noted, while the presence of one potential serious interaction was more common in patients with HAQ-DI ≥ 1.5, whose values indicate that respondents have significant limitations in performing physical activities. In a study conducted by Ma et al., the presence of interactions in patients with RA (33.6%) was significantly less than in the study (25). The results of regression analysis confirm that the total number of interactions is a parameter that statistically significantly affected the functional ability of the respondents (HAQ-DI ≥ 1.5). However, there are currently no available literature data to compare the results of the study related to the association of potential interactions and functional status of patients with RA.

Polypharmacy can be an important predictor of clinical outcomes in patients with RA and should be considered a "double-edged sword". On the one hand, given the presence of multiple comorbidities, it is inevitable, while on the other hand, the side effects and interactions of the drugs used must be taken into account. In this regard, it is of great importance to consider the impact of polypharmacy on the functional ability of patients with RA.

Conclusion

The results of this study indicate that polypharmacy was present in 75.57% of patients with RA, and the presence of one potential serious interaction was more common in patients with reduced functional capacity (HAQ-DI ≥ 1.5). Monitoring of polypharmacy in patients with RA is necessary in order to achieve optimal functional status, disease control, minimize drug interactions and side effects.

Acknowledgements

The authors would like to thank the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No: 451-03-68/2022-14/200113) for financial support.

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Originalni rad **UDC: 615.015.2:616.72-002.77**
doi:10.5633/amm.2022.0404

UTICAJ POLIFARMACIJE NA FUNKCIONALNU SPOSOBNOST OBOLELIH OD REUMATOIDNOG ARTRITISA

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Kliničke smernice lečenja reumatoidnog artritisa (RA) zasnivaju se na primeni: nesteroidnih, antiinflamatornih lekova (NSAID), konvencionalnih lekova, koji modifikuju tok bolesti (csDMARD), imunosupresiva i biološke terapije. Nažalost, veliki broj bolesnika pored RA ima i druge hronične bolesti, što zahteva dodatnu višestruku primenu lekova i često vodi ka polifarmaciji. Prema definiciji Svetske zdravstvene organizacije (SZO; World Health Organization – WHO; eng), polifarmacija predstavlja rutinsku upotrebu 5 ili više lekova, uključujući lekove sa lekarskim receptom ili bez lekarskog recepta, dijetetske suplemente i tradicionalne lekove. Cilj sprovedenog istraživanja jeste ispitivanje uticaja polifarmacije na funkcionalnu sposobnost bolesnika, koji boluju od RA, procenjenu na osnovu vrednosti HAQ-DI indeksa.

Sprovedeno retrospektivno istraživanje uključivalo je 131 bolesnika sa dijagnozom RA. U sklopu kliničkih karkteristika, pažnja je bila usmerana ka prisutnim komorbiditetima, terapiji i ukupnom broju primenjivanih lekova. Za procenu zdravstvenog stanja i funkcionalne sposobnosti primenjivan je HAQ upitnik. Dobijeni podaci analizirani su i statistički obrađeni primenom odgovarajućih softverskih i statističkih metoda.

U sprovedenom istraživanju učestvovao je 131 bolesnik, 29 bolesnika muškog i 102 bolesnika ženskog pola, prosečne starosti 60,25 godina ± 11,21 godina. Analizom prikupljenih kliničkih podataka uočeno je prisustvo komorbiditeta kod 80,15% bolesnika. Lekove koji modifikuju sintetičke bolesti primenjivalo je 88,55%, dok je na biološkoj terapiji bilo 13,74% bolesnika. Vrednosti HAQ-DI < 1,5 bile su prisutne kod 83,21% ispitanika, dok su vrednosti HAQ-DI ≥ 1,5 zabeležene kod 16.79% ispitanika. Polifarmacija je bila prisutna kod 75,57% ispitanika. Potencijalne interakcije bile su učestalije u grupi ispitika koji mogu obavljati uobičajene fizičke aktivnosti bez ograničenja ili uz blaga ograničenja (HAQ-DI < 1,5), dok je prisustvo jedne ozbiljne potencijalne interakcije bilo izraženije u grupi ispitanka sa smanjenom funkcionalnom sposobnošću (HAQ-DI ≥ 1,5).

Rezultati spovedenog istraživanja pokazuju veliku učestalost polifarmacije i posledničnih potencijalnih interakcija lekova kod bolesnika sa RA. U skladu sa tim, praćenje polifarmacije kod bolesnika koji boluju od RA neophodno je u cilju postizanja optimalnog funkcionalnog statusa, kontrole bolesti, minimiziranja interakcija i neželjenih efekta lekova.

Acta Medica Medianae 2022;61(4):24-30.

Ključne reči: *polifarmacija, reumatoidni artritis, HAQ-DI*

Original article

UDC: 616.8-009.7-083:615.212
doi:10.5633/amm.2022.0405

EXPERIENCES OF PATIENTS TREATED IN THE REANIMATION UNIT REGARDING PAIN EXPERIENCE, ADEQUATE PAIN ASSESSMENT AND PAIN ALLEVIATION

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An intensive care and reanimation unit (ICU) is a hospital ward that provides optimal care and treatment for critically ill patients. The reanimation unit is a very stressful environment for patients. Aims of the study were to evaluate and identify the pain experience of ICU patients and adequate assessment of pain. This study was conducted as a prospective, observational study involving 121 patients treated in the Reanimation Department, Emergency Center, Clinical Center of Vojvodina. All the examined patients over 18 years of age hospitalized in this Department who met inclusion criteria were interviewed at the bedside. Data were collected by using the questionnaire filled in by a doctor during their conversation with a patient. Thirty-two point two percent of patients were hospitalized from 3 to 7 days, the same percentage of patients were hospitalized for more than 7 days, while 21.5% were hospitalized 1–3 days and 14.1% for one day. Sixty-one point eight percent of the patients reported inability of speech, 14.5% of patients stated their inability of speech and not knowing who to refer to as a problem. Sixty-four point four six percent of patients received analgesic therapy soon after complaining of pain, while 4.13% of patients reported not having analgesics after complaining of pain. Eighty-four point one percent of patients received therapy intravenously. Fifty-six percent of patients answered positively and 3.5% of patients were not satisfied with prescribed analgesics. Fifty-five point three seven percent of patients had the strongest daytime pain. Many factors contributed to increased pain intensity: the extent of performed surgical treatment, surgical wound, injuries and fractures, numerous medical procedures, prolonged immobility in bed and back pain as a consequence.

Acta Medica Medianae 2022;61(4):31-39.

Key words: *pain, intensive care, reanimation, analgesic treatment*

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Introduction

An intensive care and reanimation unit (ICU) is a hospital ward that provides optimal care and treatment for critically ill patients. Critically ill patients admitted to intensive care unit (ICU) due to the nature of illness and/or injury, with impaired vital functions, require specialized treatment and continuous monitoring directed at supporting vital functions. Pain is a major problem in critically ill patients (1-5). There are multiple contributing factors that cause pain: recent diseases and injuries, trauma, routine medical procedures (3, 5, 6). The postoperative period of major surgeries is extremely painful, and pain is the most common complication during that time (2). Postoperative pain in the reanimation units causes great discomfort in patients, but critically ill patients are not able to communicate about their suffering due to continuous administration of sedatives and analgesics (3). Thus, pain relief and patients' comfort are becoming a priority, not only for ethical and humane issues, but also for providing physical, mental and social benefits (4).

The reanimation unit is a very stressful environment for patients. They face numerous stressors: pain, painful invasive and noninvasive procedures, inability to get up, constant noise, disturbance in sleeping cycle, lack of privacy, fear and anxiety regarding the treatment outcome, the absence of family members in difficult situations, crowding with unfamiliar people and so on. All of these may cause high levels of anxiety and agitation in patients. Also, disruption of normal sleep pattern may weaken the immune system, increase susceptibility

to infections, and delay wound healing and overall recovery. Pain is a significant problem in critically ill patients (1-5). Pain during nursing procedures is rather common, and even routine procedures, such as transfer of patients, are associated with significantly high pain intensity. According to some studies repositioning and turning the patient is the most painful procedure (5, 7, 8). For all these reasons, pain monitoring and management in reanimation units is considered a priority and an ethical obligation of all health professionals (9, 10).

It is necessary to humanize intensive care units (1). This concept is becoming more and more used and it is related to pointing out the importance of creating comfortable environment that meets the needs of patients and respect for the patient, not observing patient as 'a disease'. The aim is to create an environment where a patient is seen as a human being, not an object, promoting interpersonal relationship. Humanization of reanimation units is closely related to behavior of health professionals in stressful surrounding, in pain and anxiety control, and in providing better psychological and emotional comfort to patients (11).

Experience of patients treated in the reanimation unit in relation to pain experience, adequate pain assessment and pain relief, might contribute in humanizing such wards, preventing unnecessary harm being done to patients. Therefore, this study was also aimed at providing information on patients' experience in the reanimation unit in relation to pain assessment and pain management, and experience related to stressors patients were exposed to.

Aims of the study were to evaluate and identify pain experience of ICU patients and adequate assessment of pain and its alleviation as well.

Patients and methods

This study was conducted as a prospective, observational study involving 121 patients treated in the Reanimation Department, Emergency Center, Clinical Center of Vojvodina. The Reanimation Unit has 15 beds for surgical patients, but also for patients with threatened vital functions that require support. All the examined patients over 18 years of age hospitalized in this Department who met inclusion criteria were interviewed at the bedside immediately before transferring to another unit or Clinic of the Vojvodina Clinical Center, or to another clinical facility.

Data were collected by using the questionnaire (Appendix 1) filled in by a doctor during their conversation with a patient.

Appendix 1. QUESTIONNAIRE

1. Date of birth:	
2. Gender:	
3. Cause of hospitalization:	a) polytrauma b) postoperative pain c) sepsis d) other.....
4. Length of stay at Intensive Care Unit:	a) 1 day b) 1-3 days c) 3-7 days d) more than 7 days
5. Did you have any problems in expressing your pain?	a)yes b) no
6. If 'yes', what was the problem?	a) inability to speak b) I did not know who to address c) nobody listened
7. Were you asked questions about pain during your stay in the Reanimation Unit?	a) yes, several times a day b) yes, only occasionally c) no, nobody asked me about pain
8. If 'yes', what were the questions like?	a) only if I had pain or not b) using numeric pain scale (0-10) e) questions about pain characteristics d) other
9. If you were asked about pain, who asked the questions?	a) doctors b) nurses
10. When complaining of pain, did you get help?	a) yes, I received medication soon b) yes, but I waited long time to get medication c) no, I did not receive medication when I complained of pain
11. How was the medication administered?	a) intravenously b) orally
12. Did the medication received alleviate pain?	a) yes, completely b) yes, partially c) no, I did not feel any relief

13. When was the pain strongest?	a) during the day b) at night
14. Did you experience fear and anxiety during your stay in the Reanimation Unit?	a) yes b) no
15. What was most painful?	a) surgical wound b) wound dressing c) backache d) arterial and venous catheter placement e) central venous catheter placement f) probes and catheter placement g) thoracic drains placement h) maintain hygiene i) application of enema j) inflating cuffs for pressure measurement k) frequent venipunctures
16. Was there any procedure that caused pain and was not mentioned in the previous question?	a) yes b) no
17.If yes, which one?.....	

Data on age, gender, reason for hospitalization and length of hospital stay were entered. Then questions about pain were asked, problems with reporting the pain level, followed by questions on health care professionals in relieving the patients' pain, as well as the efficacy of applied treatment. The last set of questions was related to pain during routine medical procedures. The questionnaire used in this study was a redesigned questionnaire from a study referred to as a reference No. 1, Chapter 7.

The study enrolled all the patients who were conscious, coherent, and oriented in time and space at the moment of transfer.

Patients with cognitive disorders and those unable to answer the questions for any reason were excluded from the study. Patients who withdrew from the research during the interview were also excluded from the study. Participation in the study was on voluntary basis.

Collected data were coded and entered into a database in Excel. The database was specially designed for the needs of research. During the statistical data processing, descriptive statistics were calculated: frequency, mean value, median value, median, standard deviation, the minimum, the maximum, percentage. The results of the study are shown in tabular form.

Microsoft Excel 2007 and software package Statistica 13 (StatSoft Inc., Tulsa, OK, USA) with the University License, University of Novi Sad, were used for statistical analysis.

Results

A total of 121 patients were interviewed in this study. Mean age of patients was 58.6 ± 13.1 years, in the range from 33 to 86 years. Fifty percent of patients were under the age of 58 years. Forty-six point three percent were males and 53.7% females, as illustrated in Table 1.

The majority of patients were admitted to the Reanimation Unit postoperatively (39.7%), other medical causes were present in 26.4% of cases, then polytraumatized patients in 18.2% of cases and sepsis as a reason of hospitalization was present in 15.7% patients. Results related to causes of hospitalization are shown in Table 2.

Table 1. Mean age of study participants

	N	Average	Median	Minimum	Maximum	Standard deviation (SD)
Age	121	58.6	58.0	33.0	86.0	13.1

Table 2. Causes of hospitalization

Cause of hospitalization	Number	Percentage
1. polytrauma	22	18.2
2. postoperatively	48	39.7
3. sepsis	19	15.7
4. other	32	26.4

As for the length of hospital stay in the Reanimation Unit, 32.2% of patients were hospitalized from 3 to 7 days. The same percentage of patients were hospitalized for more than 7 days, while 21.5% were hospitalized 1-3 days, and 14.1% of patients for one day.

When asked about problems in communicating about pain, 45.5% of patients reported that there had been a problem, as illustrated in Table 3.

When asked about the type of problem, most of the patients reported inability of speech (61.8%), 14.5% of patients stated their inability of speech and not knowing who to refer to as a problem, as shown in Table 4.

When asked about being questioned about pain during their stay in the Reanimation Unit, majority of patients answered positively (57.8%), while 5.8% of patients answered they had never been asked about pain during their stay in the Reanimation Unit. Methods of pain history taking are shown in Table 5.

The study showed that the greatest number of patients were asked about the pain equally by nurses and doctors (52.7%), but only 8 patients reported being asked about pain only by nurses (7.1%).

Majority of patients (64.46%) received analgesic therapy soon after complaining of pain, while 5 patients reported not having analgesics after complaining of pain (4.13%), as seen in Table 6.

Table 3. Patients' problems in expressing pain level

Did you have any problems in expressing your pain?	Number	Percentage
1. Yes	55	45.5
2. No	66	54.5

Table 4. Patients' problems related to expressing the pain

If 'yes', what was the problem:	Number	Percentage
1. Inability of speech	34	61.8
2. I did not know who to speak to	4	7.3
3. Nobody listened to me	3	5.5
4. Inability of speech and nobody listening	5	9.1
5. Inability of speech and not knowing who to talk to	8	14.5
6. Inability of speech, not knowing who to talk to and nobody listened	1	1.8

Table 5. The way pain history was taken

How were the questions about pain asked?	Number	Percentage
1. By asking only if I had pain or not	50	42.7
2. By using numeric pain scale (0-10)	15	12.8
3. By asking about pain characteristics	7	6.0
4. Other	3	2.6
5. By using numeric pain scale (1-10); questions on pain characteristics	26	22.2
6. By asking only if I had pain or not; questions on pain characteristics	4	3.4
7. By asking only if I had pain or not; questions on pain characteristics	1	0.9
8. By asking only if I had pain or not; using numeric pain scale (0-10)	8	6.8
9. By asking only if I had pain or not; using numeric pain scale; asking questions on pain characteristics	3	2.6

Table 6. Time interval in administering analgesic therapy

When complaining of pain, did you get the treatment:	Number	Percentage
1. Yes, I received medication soon	78	64.46
2. Yes, but I waited long time before receiving the medication	33	27.27
3. No, I did not get medication after complaining of pain	5	4.13

As it has been expected, the greatest number of patients received therapy intravenously (84.1%).

Regarding easing the pain after administered therapy, 56% of patients answered positively, and 3.5% of patients were not satisfied with the prescribed analgesic treatment.

In relation to time of the day when intensity of pain was the strongest, most of the patients said that the pain had been the strongest during daytime (55.37%), as shown in Table 7.

When asked about the feelings of fear and anxiety while in the Reanimation Unit, most patients (58.3%) answered positively.

For most of the patients back pain was dominant pain (47.1%) as a consequence of prolonged immobility, followed by surgical wound (38.8%), central venous catheter placement (30.6%), frequent venipunctures (26.5%), and nursing care as well (24.8%). These results are given in Table 8.

Most patients could not recollect any other procedure that caused pain during their stay in the Reanimation Unit. According to those who reported it (13.22%), the most painful procedure was aspiration (18.75%), followed by uncomfortable antidecubitus mattresses and transfer from the bed to stretcher (12.5%), as shown in Table 9.

Table 7. Time interval in having pain

When was the pain more intense:	Number	Percentage
1. During the day	67	55.37
2. At night	36	29.75
3. Both during the day and at night, regardless the time of the day	15	12.39

Table 8. Contributing factors to most intense pain in patients

What was most painful experience during your stay in the Reanimation Unit?	Number	Percentage
1. Surgical wound	47	38.8
2. Wound dressing	20	16.5
3. Backache	57	47.1
4. Arterial and venous lines placement	24	19.8
5.Central venous catheter placement	37	30.6
6. Probes and catheter placement	30	24.8
7. Thoracic drain placement	26	21.5
8. Hygiene maintenance	30	24.8
9. Receiving enema	20	16.5
10. Inflation of the cuffs for pressure measurement	12	9.9
11. Frequent venipunctures	32	26.5

Table 9. Procedures that caused pain in patients

What procedure caused your pain?	Number	Percentage
Extubation	1	6.25
The presence of urinary catheter	1	6.25
Aspiration, coughing	3	18.75
Antidecubitus mattress	2	12.5
Turning to the side	1	6.25
Transfer from bed to stretcher	2	12,5
Decubitus wounds	1	6,25
Nursing care, physical therapy	1	6.25
Drain removal	1	6.25
Pain while swallowing	1	6.25
Getting out of bed	1	6.25
Cold showers	1	6.25
Total:	16	

Discussion

The aim of the study was to investigate patients' experience in the Reanimation Unit regarding pain experience, adequate pain assessment and pain relief. Majority of patients in this study were females (53.7%). Mean age of the patients was 58.6 13.1 years, ranging from 33 to 86 years. Fifty percent of patients were under 50 years of age.

Fear and anxiety were most present in the group of patients who were in the Reanimation Unit from 3 to 7 days (40%), then in the group hospitalized for more than 7 days (35.7%). Fear and anxiety during the hospital stay in the Reanimation Unit were present in 58.3% of cases in comparison to the total number of patients. Fear and anxiety are consequences of pain, too much noise caused by alarms, telephones, shouting, and lack of empathy from the health-care team (6). All aforementioned result in sleep disturbances and disruption of circadian rhythms, which might lead to the development of delirium, especially in elderly patient (6, 7).

Pain is a subjective phenomenon. Its intensity is influenced by social and cultural beliefs, personal emotions, mental status, and understanding of pain and it is related to positive or negative expectations from patients (12, 13). Pain is expected and accepted by some people, but not by others (14-16). Different people under similar or identical circumstances may experience pain in a completely different way (17). Although most patient reported not having problems in expressing pain, 45.5% of patients reported some kind of a problem. In most cases, patients mentioned inability to speak (61.8%), followed by inability to speak associated with the fact that they did not know who to speak to (14.5%).

According to the study results, 57.8% of patients said they had been asked about the pain several times a day, while 5.8% of patients reported nobody at all had asked them about the pain during their stay in the Reanimation Unit. The patients answered the question of how they were asked about the pain during their hospitalization as follows: most patients (42.7%) were asked a simple question of whether they were having any pain or not, while 22.2 % of patients were asked to answer the questions about pain by using numeric pain scale; they were also asked to describe the pain. Such an approach by asking simple question whether the pain is present or not is not in accordance with the literature recommendations which suggest the importance of pain assessment scale in order to achieve a more efficient therapy and treatment outcomes (18-20). The scale is used to define pain, to quantify pain intensity, to uniformly monitor and provide better treatment. The use of scales would certainly contribute to better pain intensity tracking and to better communication among health-care professionals by having a more clear perception of pain syndrome and response to administered analgesic treatment. It would be ideal to use a uniform pain assessment scale, but it is often not possible, considering diversities of patients.

The questions about pain were asked both by doctors and by nurses (52.7%), while pain assessment was performed by doctors (40.2%). Patients consider health-care workers, especially nurses, as protective persons who take care about their needs during the period of suffering and vulnerability (21).

Majority of patients reported to have received adequate analgesic treatment soon after making a complaint about pain (64.46%), while 5 patients revealed they had not received any medications after complaining of having pain (4.13%). It is difficult to assess pain objectively due to its subjective and multidimensional nature. That is why so called verbal self-report is of key importance. Pain is often underestimated, especially by doctors and nurses as well, since pain relief is often not a primary concern, resulting in the lack of prevention measures and reaction only at the moment of patient's complain about pain (22). In 84.1% of cases, analgesics are administered by intravenous injections.

Most of the patients reported that the analgesic therapy they had received was entirely satisfactory (56%), while 47% of them said that received analgesics resulted in a partial pain relief only. Four patients had pain even after administered therapy. Pharmacotherapy for pain management is necessary in postoperative period (23). Prescribed drugs have proved effective in this study since 56% of patients reported complete pain relief. This result is in accordance with previous studies that conducted research on pain control in patients in the Reanimation Unit, when the pain was of moderate intensity providing timely strong analgesics were given (12, 13). Pharmacotherapy is the best way of pain management, but other measures are also available, such as relaxation and distraction techniques, regardless of patient-controlled analgesia. These techniques are especially useful in case of painful procedures, such as wound dressing, turning the patient, care and physical therapy, or while waiting for the effect of already administered analgesics. Being an empathetic health professional and recognizing patient's pain, contribute to minimizing pain (24).

Patients mostly suffer from pain during daytime (55.37%), while 12.39% of patients had pain during the day and at night as well. This is related to numerous painful invasive medical procedures performed during the day, to patients' transport, diagnostic procedures, physical and respiratory therapy.

The most dominant pain in patients treated in the Reanimation Unit was back pain, even in 47.1% of patients. According to Gelinias et al. studies, pain increases morbidity and mortality rate in cardiosurgical patients. Even 62% of cardiosurgical patients identify body movement as the main pain trigger. This is in accordance with our study as well. Other studies conducted after thoracic and abdominal surgeries also showed that the pain was stronger after body movements and respiratory physical therapy (15, 16). Immobilization aiming at the reduction of pain is a very dangerous method of relieving the pain. Immobilization may be considered as an important pain indicator (25). The second worst pain occurred as a consequence of surgical wound (38.8%). As for painful procedures, placement of central venous catheter was the most painful procedure (30.6%); frequent peripheral venipuncture was a painful procedure in (26.5%) of patients. It is

interesting that nursing care procedures and hygiene maintenance resulted in severe pain in 24.8% of patients.

Patients also reported the following painful procedures: pain from antidecubitus mattress, lateral turning, endotracheal aspiration, breathing exercises, coughing, drains removal, and decubitus ulcers. According to the patients who reported painful procedures (13.22%), the most painful procedure was aspiration (18.75%), followed by uncomfortable anti decubitus mattress, and transfer from bed to stretcher (12.5%). In a study by Arroyo et al. (26), it was confirmed that 30% of patients identified endotracheal aspiration as the most painful procedure. It is also interesting that a study by Aslan et al. showed that antidecubitus air mattress caused pain in 6.7% of patients, a result that no other studies have reported so far. In our study, two patients reported pain because of antidecubitus mattress (1.65%). Such results are in accordance with the results of studies that investigated procedu-ral pain in the Reanimation Units (27).

Conclusion

Patients hospitalized in the Reanimation Unit were completely satisfied with pain control and medication effectiveness. However, many factors contributed to increased pain intensity: the extent of performed surgical treatment, surgical wound, injuries and fractures, numerous medical procedures, prolonged immobility in bed and back pain as a consequence.

Pain is the fifth vital sign, so the pain intensity should be assessed at the same time as other vital signs are checked, but also in emergency situations when invasive medical procedures, nursing care and physical therapy are performed.

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Originalni rad **UDC: 616.8-009.7-083:615.212**
doi:10.5633/amm.2022.0405

ISKUSTVA BOLESNIKA LEČENIH NA ODELJENJU REANIMACIJE, VEZANA ZA DOŽIVLJAJ BOLA, ADEKVATNOST PROCENE I KUPIRANJA BOLA

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Jedinica intenzivne nege i reanimacije (JIL) je bolničko odeljenje u kojem se pruža optimalna nega kritičnim bolesnicima i vrši se lečenje kritičnih bolesnika. Odeljenje reanimacije je vrlo stresno okruženje za bolesnike. Ciljevi ovog istraživanja bili su procena i identifikacija iskustva bola kod bolesnika na intenzivnoj nezi i adekvatna procena bola. Ova studija sprovedena je kao prospektivna, opservaciona studija na 121 bolesniku, lečenom na Odeljenju reanimacije Urgentnog centra Kliničkog centra Vojvodine. Svi pregledani bolesnici, koji su zadovoljili kriterijume uključivanja u studiju, hospitalizovani na ovom odeljenju, bili su stariji od 18 godina i intervjuirani su uz krevet. Podaci su prikupljeni korištenjem upitnika, koji je lekar ispunio tokom razgovora s bolesnikom. U trajanju od 3 do 7 dana hospitalizovano je 32,2% bolesnika, isti procenat bolesnika hospitalizovan je u trajanju dužem od 7 dana, dok je 21,5% hospitalizovano od jednog do 3 dana, a 14,1% na jedan dan. Nemogućnost govora navelo je 61,8% bolesnika, 14,5% bolesnika kao problem navelo je nemogućnost govora i zbunjenost po pitanju toga kome bi se obratili. 64,46% bolesnika primilo je analgetsku terapiju ubrzo nakon žalbe na bol, dok je 4,13% bolesnika izjavilo da ne uzima analgetike i nakon što su se žalili na bol. 84,1% bolesnika primilo je terapiju intravenozno. Na pitanja o zdovoljstvu lekom, potvrdno je odgovorilo 56% bolesnika, dok 3,5% bolesnika nije bilo zadovoljno propisanim analgeticima. Najjači dnevni bol imalo je 55,37% bolesnika. Pojačanju intenziteta bola doprineli su brojni faktori: opseg izvedenog hirurškog lečenja, hirurška rana, povrede i prelomi, brojni medicinski zahvati, dugotrajna nepokretnost u krevetu i, kao posedica toga, bol u leđima.

Acta Medica Medianae 2022;61(4):31-39.

Ključne reči: *bol, intenzivna nega, reanimacija, analgetska terapija*

Original article

UDC: 616.346-002-072.1-089
doi:10.5633/amm.2022.0406

LAPAROSCOPIC VERSUS OPEN APPENDECTOMY FOR IN THE TREATMENT OF ACUTE APPENDICITIS: OUR EXPERIENCE

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Acute appendicitis is one of the most common urgent abdominal interventions. Open appendectomy has been a standard procedure for acute appendicitis for more than 100 years. However, in the last 20 years, after many studies, laparoscopic appendectomy has become a gold standard in solving acute appendicitis. The main goal of our study was to compare results of open and laparoscopic appendectomies with regard to in-hospital stay, time of operation, postoperative complications and postoperative pain.

All patients underwent open or laparoscopic appendectomy in the Center of Minimally Invasive Surgery and Emergency Center of the University Clinical Center Niš, Serbia in the period of one year. A total of 126 patients were enrolled and submitted to retrospective analysis.

One hundred and twenty-six patients who underwent laparoscopic or open appendectomy surgery were retrospectively analysed. A laparoscopic appendectomy was performed in 58 patients, while 68 patients underwent an open appendectomy. Groups were demographically similar and there was no significant difference between the age structure and gender distribution ($t = 0.927$; $p = 0.057$). Average height ($p = 0.123$), weight ($p = 0.200$) and BMI ($p = 0.425$) were mostly similar. Previous surgical operations were more common in patients with open appendectomy, but with no statistical significance ($p = 0.141$). Percentage of patients with WBC > 10 were the same in both groups ($p = 0.927$).

Diabetes mellitus was more common in patients with open appendectomy, but with no statistical significance ($p = 0.563$). Acute and perforated appendicitis were similar in both groups ($p = 0.490$).

Average time of operation was the same in both groups ($p = 0.751$). Number of days of in-hospital stay was shorter in patients who underwent laparoscopic appendectomy ($p < 0.001$).

The analysis of administration of parenteral and oral analgesics showed that postoperative pain was less in the group of patients who underwent laparoscopic appendectomy than in the group of patients with open appendectomy.

There was no statistically significant difference with respect to postoperative complications between two groups ($p < 0.001$).

The treatment of appendicitis by using laparoscopic surgery in comparison to open approach provides a better result in terms of duration of hospital stay, recovering time, postoperative complications and postoperative pain.

Acta Medica Medianae 2022;61(4):40-47.

Key words: *open appendectomy, laparoscopic appendectomy, acute appendicitis*

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Introduction

Acute appendicitis is the most common abdominal emergency worldwide, and it is the most common cause of abdominal surgeries in all the age groups (8).

Approximately 7-10% of the general population develops acute appendicitis with the maximal incidence in the second and third decades of life (9).

Appendicitis has an overall lifetime risk of 8.6% in men and 6.7% in women (10, 11).

A definitive preoperative diagnosis of acute appendicitis is still a challenge and a possibility of

appendicitis must be considered in any patient presenting with an acute abdomen (12, 13)

Since its first description by McBurney in 1894, open appendectomy has become the procedure of choice for acute appendicitis (14).

For more than a century, open appendectomy has been the gold standard for treating patients with acute appendicitis, but the efficiency and superiority of laparoscopic approach compared to the open technique is the subject of many studies nowadays (15-16).

With the introduction of minimally invasive endoscopic surgery, laparoscopic appendectomy, which was first introduced by Kurt Semm, a German gynaecologist in 1981 (10), has become increasingly popular and is claimed to be more safe and superior to open appendectomy in terms of hospital stay, postoperative pain, wound complications, diagnostic abdominal exploration, return to normal activities and cosmetic result (17, 18).

The aim of our study was to compare results of open and laparoscopic appendectomies in regard to in-hospital stay, time of operation, postoperative complications and postoperative pain.

Materials and methods

A retrospective study of patients with the diagnosis of acute appendicitis was conducted.

This retrospective study was carried out on the basis of medical data of patients who were subjected to open or laparoscopic surgery for acute appendicitis in the period of one year. Patients underwent surgery in the Emergency Center and Center of Minimally Invasive Surgery of the University Clinical Center Niš, Serbia.

Data were collected regarding demographics, preoperative assessment, intraoperative findings, operative time, length of hospital stay, and occurrence of postoperative complications, including wound infection, intraperitoneal collection, paralytic ileus.

The diagnosis of appendicitis was determined by clinical examination (pain after deep palpation in right iliac fossa, status febrilis, leukocytosis, nausea,…). If we had had any doubt in establishing the diagnosis, the next step would have involved abdominal ultrasound or CT. In addition, an increase in white blood cell count and increase in C reactive protein were indicators for an acute appendicitis.

All patients received general endotracheal anaesthesia. In majority of cases, there was no need for nasogastric tube insertion.

Irrigation of abdominal cavity and drainage placement depended on surgeon's decision and were used mostly in perforation. All specimens were sent for histopathology.

All patients subjected to open and laparoscopic appendectomies received antibiotics and analgesics intravenously. Commonly, if the drain bag was empty, it was removed one day after surgery.

The oral feeding of patients whose peristalsis recovered was started after 2 or 3 days. It was started with fluids and liquid diet. On discharge, the patient underwent pain control, had normal body temperature and good physical condition.

In this study, the duration of performing surgery was measured from the first skin incision to the last skin stich excluding the time of anaesthesia and preparation.

The number of hospital days were counted as nights spent in hospital after surgery. Symptoms of wound infection were determined as wound leakage of consistent purulent content. In that case, red tissue margins could be seen around wound along with wound warmness. Prolonged paralytic ileus could be present in some patients and appeared as lack of bowel peristalsis within 72 h after surgery.

The same team of surgeons performed all surgeries.

Results of statistical data analysis are shown in tables. Statistical data were analysed by using SPSS software package version 22.

Standard statistical methods for quantitative and qualitative analysis were used: absolute numbers, relative numbers (%), arithmetic mean ( ), standard deviation (SD), mediana (Me), interquartile range (Iq). Probability distribution was analysed with Kolmogorov-Smirnov test.

For comparison of groups, Student-t test and Mann Whitney U test were used.

For testing statistical difference of absolute frequencies among samples Chi square test was used.

Statistical hypotheses were tested on the level of significance for risk of $\alpha = 0.05$.

Statistical difference between samples was considered significant at $p < 0.05$.

Results

Our study included 126 patients who underwent appendectomy surgery. Gender distribution was 70 (55.6%) male patients and 56 (44.4%) female patients.

Age structure was 43.38 ± 17.48 age, where the youngest patient was 18 years old and the oldest patient was 85 (median age 37 years) There was no significant difference between age structure and gender distribution, $p = 0.780$ (Table 1).

Open appendectomy was performed in 68 (53.96%) patients, while laparoscopic appendectomy was done in 58 (46.04%) patients.

Intraoperative and pathological findings of phlegmonous appendix were recorded in 90 (71.4%) patients, while gangrenous appendix with perforation was seen in 36 (28.6%) patients. There were no significant differences regarding intraoperative and pathohistological findings between the groups.

Average time of open appendectomy was 32 minutes. Average time of in-hospital stay was 5.1 days, with minimal in-hospital stay of 2 days and maximum in-hospital stay of 14 days (Table 2).

Table 1. Age, gender and anamnesis structure in open appendectomy

	LA	OA
No of patients	58	68
Sex (male/female)	33/25	37/31
Age (years)	35.05 ± 15.93	40.95 ± 17.75
Weight (kg)	79.77 ± 15.23	75.63 ± 14.32
Height (cm)	175.16 ± 10.48	172.82 ± 9.52
BMI	25.88 ± 3.52	25.33 ± 3.68
Previous surgeries n (%)	7 (12.1)	15 (22.1)
WBC > 10 n (%)	50 (86.2)	59 (86.8)
Diabetes mellitus n (%)	5 (8.6)	8 (11.8)
Acute appendicitis n (%)	41 (70.7)	49 (72.1)
Perforated appendicitis n (%)	14 (24.1)	18 (26.5)

LA - Laparoscopic appendectomy; OA - open appendectomy;
WBC - white blood cell count; BMI - body mass index

Table 2. Clinical data for the laparoscopic appendectomy (LA) and open appendectomy (OP) groups

Clinical data	LA	OA	p-value
Operating time (min)	32.63 ± 15.96	31.76 ± 14.50	0.751
Hospital stays (days)	4.12 ± 1.09	5.97 ± 1.26	< 0.001
CRP	42.46 ± 52.71	55.38 ± 67.39	0.242
WBC	13.55 ± 3.49	13.33 ± 3.14	0.706
Bowel movements (first day)	25	5	< 0.001
Bowel movements (second day)	33	66	< 0.001

The results of bowel movements were as follows: the highest number of patients, i.e. 96 (76.2%) showed bowel movements on the second postoperative day, on the first postoperative day, bowel movements were present in 30 (23.8%) patients. Most of the patients who had laparoscopic appendectomy showed bowel movements on the first postoperative day. There was a significant difference between two groups, it showed faster bowel movements in patients treated by laparoscopic approach ($p < 0.001$).

The most frequent postoperative complication was wound infection present in 5 (3.96%) patients. Prolonged postoperative ileus was present in 10 (7.9%) patients, and 2 (1.6%) patients had paralytic ileus (Table 3). All wound infection were in patients after open appendectomy, while there were only six abdominal abscesses as a complication of laparoscopic appendectomy.

Table 3. Postoperative complications

Clinical data	LA n (%)	OA n (%)	p-value
Abdominal abscess	6 (10.3)	4 (5.9)	0.551
Paralytic ileus	0 (0.0)	2 (2.9)	0.499
Wound infections	0 (0.0)	5 (7.4)	0.061
Total	6 (10.3)	11 (16.2)	0.435

As for postoperative pain, analysing the usage of parenteral and oral analgesics showed that the patients subjected to laparoscopic appendectomy had less pain than the patients subjected to open appendectomy did. The group of patients treated with laparoscopic appendectomy was administered parenteral analgesics (doses/day) 1.4 ± 0.5 and oral analgesics (doses/day) 2.00 ± 2.26. The group of patients treated with open appendectomy was administered parenteral analgesics (doses/day) 1.0 ± 0.4 and oral analgesics 1.79 ± 1.9 (Table 4).

Table 4. Parenteral and oral analgesics use during hospital stay

Clinical data	LA	OA	p-value
Parenteral analgesics (doses/day)	1.4 ± 05	2.00 ± 2.26	0.049
Oral analgesics (doses/day)	1.0 ± 0.4	1.79 ± 1.9	0.002

Univariate regression analysis (Table 5) showed that age (OR = 1.151; p=0.001), WBC (OR = 1.471; p < 0.001), CRP (OR = 1.022; p < 0.001), surgery duration (OR = 1.078; p < 0.001) and hospital stay (OR = 2.017; p = 0.001) could be risk factors for complications.

However, when we included all these variables in multivariable model, there was no statistical significance.

Table 5. Univariate and multivariate regression analysis for postoperative complications
OR- Odds ratio; CI-Confidence interval for OR

Factor	Univariate regression				Multivariate regression model			
	OR	95% CI		p	OR	95% CI		p
		Lower	Upper			Lower	Upper	
ex	0.644	0.222	1.865	0.417				
Age	1.151	1.021	1.082	0.001	1.151	1.021	1.082	0.255
Type of operation	1.673	0.578	4.844	0.343				
BMI	1.073	0.932	1.235	0.330				
Previous operations	2.255	0.704	7.226	0.171				
DM	1.188	0.239	5.893	0.833				
Le	1.471	1.207	1.793	< 0.001	1.294	0.904	1.852	0.160
CRP	1.022	1.012	1.032	< 0.001	1.012	1.000	1.024	0.051
Operating time	1.078	1.041	1.116	< 0.001	1.030	0.975	1.089	0.289
Hospital stays	2.017	1.343	3.030	0.001	1.384	0.748	2.558	0.301

Discussion

Acute appendicitis is the most common abdominal emergency condition worldwide.

Nowadays, many studies compare open to laparoscopic appendectomy in relation to advantages and possible complications. Several studies (15, 19-25) have described laparoscopic appendectomy as a more safe procedure. Patients were back to work very soon, sooner than after open appendectomy. Further, there were less wound infections due to small skin incision versus McBurney incision. Several studies underlined this approach as better, because of the clear laparoscopic exploration and abdominal inspection in finding some other surgical problem (camera position, flexible movement, far distance cavity approach, camera view extension) (26, 27). In some older studies, few authors claimed that there were not any benefits of using laparoscopic versus open appendectomy (9, 28-31).

Systematic review of meta-analyses of randomized controlled trials comparing laparoscopic versus open appendectomy concluded that both procedures were safe and effective for the treatment of acute appendicitis (30).

The aim of this study was to retrospectively evaluate the results such as time of operation, postoperative hospital stays, and postoperative complications of laparoscopic appendectomy in the treatment of acute appendicitis in comparison with the open approach.

Length of hospital stay represents a critical factor that directly influences the economy and the well-being of the patient. We found that hospital stay was significantly shorter in the laparoscopic group ($p < 0.001$) with a concomitant earlier bowel movement in patients managed laparoscopically, leading to earlier feeding and discharge from hospital. Our findings are in agreement with several studies that demonstrated a significantly shorter hospital stay after the laparoscopic approach (24, 32, 33).

By analysing the immediate postoperative recovery, our study showed that in majority of the laparoscopically treated patients peristalsis occurred faster, and oral feeding was initiated earlier. These results, which indicate a statistically significant difference between the two groups of patients, definitely confirm the advantage of the laparoscopic approach in resolving acute appendicitis.

Duration of operation was very close, in laparoscopic (32.63 ± 15.96) and open approach (31.76 ± 14.50).

When we calculate the duration of surgery, our conclusion is that there was no much difference between the two approaches. Some studies show that the time for laparoscopic appendectomy is longer (34). This depended on the case difficulty, surgeon's skills and experience.

Today, most of surgeons in the beginning of their careers have many training hours which provide them with good laparoscopic skills. The result of this, according to several meta-analyses, is the reduction in operation time compared to early open technique (35, 36).

Almost all studies found that patients who underwent laparoscopic appendectomy had less postoperative pain and discomfort. These randomized prospective studies usually used visual analogue scales and other tabulated doses or days of narcotic use to record pain and pain control. Since our study was a retrospective one, we were not able to use visual analogue scales to assess the degree of pain and discomfort.

Great variability exists in the literature (27) partly due to heterogeneity in definition and assessment of pain and variety of analgesics.

However, our findings on dose usage of analgesics postoperatively retrieved from patients' medical records are in agreement with studies which showed that patients who had laparoscopic appendectomy needed significantly less analgesia, suggesting that they suffered less pain and discomfort (37, 38).

Considering postoperative complications given in Table 4, there was a statistical difference in wound infection between patients who underwent laparoscopic versus open appendectomy.

It can be due to smaller skin incision in laparoscopic approach.

In addition, our study showed difference of time of paralytic ileus between two different techniques. All patients who had laparoscopic appendectomy, has faster recovery and peristaltic function getting to normality (majority of patients regained bowel movements on the first postoperative day while the patients with open approach recovered bowel movements on the second postoperative day). Further, prolonged postoperative ileus was seen in patients with open appendectomy. We can conclude that minimal operative trauma, less pain, lack of wound infection, less number of hospital days give this results (36, 39).

Wound infection can be a factor of long hospital stay, late hernia appearance in incision place, increasing recovering time and finally, cost benefit of operation. Fewer cases of wound infection are a big benefit of laparoscopic appendectomy (36). In our study, there were no wound infections after performing laparoscopic techniques.

The use of endobag, and avoiding direct contact between the remaining appendix and surrounding tissue, especially the skin, are reasons for less wound infection.

There are still some studies claiming there is a bigger risk for intra-abdominal abscess after performing laparoscopic appendectomy techniques versus open approach (7, 23).

In addition, there were claims that several factors could contribute to the spread of bacterial infection into abdomen: carbon dioxide insufflation, extensive use of surgical irrigation, and detached appendix abdominal manipulations (40, 41).

The results of our study showed that there were no significant differences in regard to abscess formation between the two techniques.

Conclusion

This study presents laparoscopic appendectomy approach as a gold standard in treatment of acute appendicitis. Also, this technique provides better cost-benefit for the patient and our social and health system. Less hospital days, less postoperative pain, reduced number of wound infection, reduced number of postoperative hernias at incision sites, are facts why we should perform laparoscopic approach instead of open appendectomy.

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Originalni rad

UDC: 616.346-002-072.1-089
doi:10.5633/amm.2022.0406

LAPAROSKOPIJA NASPRAM OTVORENE APEDEKTOMIJE U TRETMANU AKUTNOG APENDICITISA – NAŠE ISKUSTVO

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Akutni apendicitis je jedno od najčešćih abdominalnih stanja koji zahtevaju hiruršku intervenciju. Više od jednog veka otvorena apendektomija je bila zlatni standard kao bezbedna i efikasna procedura za tretman akutnog apendicitisa. U mnogim studijama laparoskopska apendektomija je dokazana kao bezbedna i superiorna metoda . Cilj našeg rada je poređenje rezultata laparoskopske i otvorene apendektomije u smisku trajanja operacije, intrahospitalnog boravka, postoperativnih komplikacija i postoperativnog bola.

Svi bolesnici koji su imali otvorenu ili laparoskopsku apendektomiju u periodu od godinu dana u Centru za minimalno invanzivnu hirurgiju i u Urgentnom centru Univerzitetskog Kliničkog centra u Nišu. Ukupan broj od 126 bolesnika je uključen u retrospektivnu studiju.

Istraživanje je uključilo 126 bolesnika kod kojih je izvršena apendektomija. Laporaskopska apendektomija je urađena kod 58 bolesnika, a otvorena apendektomija kod 68 bolesnika. Prema polnoj distribuciji ispitivane grupe su bile homogene (p = 0,780). Bolesnici od kojih je urađena laporaskopija su nešto mlađi u poređenju sa bolesnicima sa otvorenom apendektomijem, ali bez statističke značajnosti (p = 0,057). Prosečna visina (p = 0,123), težina (p = 0,200) i BMI (p = 0,425) su ujednačeni u obe ispitivane grupe.

Prethodne hirurške operacije su zastupljenije kod OA, ali bez statističke značajnosti (p = 0,141). Procenat bolesnika sa vrednostima WBC > 10 je isti u obe ispitivane grupe (p = 0,927).

Dijabetes mellitus je češći kod pacijenata sa OA, ali bez značajne razlike (p = 0,563).

Akutni i perforirani apendicitisi su podjenako zastupljeni u obe ispitivane grupe (p = 0,490).

Trajanje operacije se nije značajno razlikovalo između ispitivanih grupa (p = 0.751). Hospitalizacija je značajno kraća kod bolesnika kod kojih je urađena laporaskopska apendektomija (p < 0.001).

Tretman akutnog apendicitisa laparoskopskom hirurgijom u poređenju sa otvorenim pristupom daje bolje rezultate u smislu intrahospitalnog boravka, vremena oporavka, postoperativnih komplikacija i postoperativnog bola.

Acta Medica Medianae 2022;61(4):40-47.

Ključne reči: *otvorena apendektomija, laparoskopska apendektomija, akutni apendicitis*

Review article

UDC: 615.214.24:616-083
doi:10.5633/amm.2022.0407

USE OF DEXMEDETOMIDINE IN AN INTENSIVE CARE UNIT

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A critical disease requiring intensive treatment represents a very stressful event. The factors preceding the admission to an intensive care unit (ICU) are life-threatening conditions, trauma or a very complex surgery, which by themselves induce a strong physiological reaction. Sedatives and analgesics are among the drugs most frequently used in ICUs. Their use aims at increasing comfort, reducing stress response and facilitation of diagnostic and therapeutic procedures. It has been confirmed that pain, oversedation and delirium are significant causes of distress in patients in ICUs and are associated with increased morbidity and mortality. The term "ICU triad" describes the close association of pain, agitation and delirium, as well as the approach to their management. The 2013 and 2018 guidelines for analgesia and sedation in the critically ill recommended the use of midazolam only for short-term sedation, lorazepam for long-term sedation, and propofol for patients in whom intermittent waking up is planned. A new version of the guidelines has given precedence to non-benzodiazepine sedatives such as dexmedetomidine. Dexmedetomidine produces a unique sedation pattern, markedly different in comparison to all other sedative drugs. The patients sedated with this drug easily establish contact, respond to verbal stimulation, communicate and cooperate with ICU staff, and after the contact is established they achieve good results at attention tests.

Acta Medica Medianae 2022;61(4):48-53.

Key words: *dexmedetomidine, sedation, intensive care unit, delirium*

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Introduction

A critical disease requiring intensive treatment represents a very stressful event. The factors preceding the admission to an intensive care unit (ICU) are life-threatening conditions, trauma or a very complex surgery, which by themselves induce a strong physiological reaction. Therapeutic interventions, especially mechanical, accompanied by environmental factors in intensive care units, act as a powerful cause of discomfort in critically ill patients. As part of the efforts to control their hemodynamic status, many of these patients receive inotrope and vasopressor support with adrenaline, noradrenaline and dopamine, which, being stress hormones, may increase the intensity of stress reaction.

Sedatives and analgesics are among the drugs most frequently used in ICUs. Their use aims at increasing comfort, reducing stress response and facilitation of diagnostic and therapeutic procedures (1).

It has been confirmed that pain, oversedation and delirium are significant causes of distress in patients in ICUs and are associated with increased morbidity and mortality. The term "ICU triad" describes the close association of pain, agitation and delirium, as well as the approach to their management (2). Consequently, sedatives should be administered only when specific pharmacological and nonpharmacological strategies aimed at pain and delirium management are employed.

Although there have been many randomized studies comparing different effects of sedatives, it could not be concluded that any of the agents is superior to any of the other agents. The choice of a sedative depends on the sedation indication in each individual patient. Essential is a thorough knowledge of pharmacodynamic and pharmacokinetic properties of sedative drugs, such as context-sensitive

half-time, metabolic pathways and presence of active metabolites and adverse effects. The choice should be guided by the patient's clinical condition, possible comorbidities and chronic therapy, as well as the presence of organic dysfunctions.

The 2013 and 2018 guidelines for analgesia and sedation in the critically ill recommended the use of midazolam only for short-term sedation, lorazepam for long-term sedation, and propofol for patients in whom intermittent waking up is planned (3).

The ABCDEF bundle (assess, prevent, and manage pain; both spontaneous awakening and breathing trials: choice of analgesia and sedation; delirium assess, prevent, and manage; early mobility and exercise; family engagement/empowerment) aims to promote practice where patients are more awake, cognitively engaged, and physically active (4).

A new version of the guidelines has given precedence to non-benzodiasepine sedatives such as dexmedetomidine.

Dexmedetomidine

While the agonists of γ-aminobutyric acid receptors are the most frequently used sedative agents in ICUs, the development of novel agents increased the number of alternative drugs. Dexmedetomidine is a selective α2-receptor agonist with sedative, analgesic and sympatholytic properties. It is a more potent, more selective and more specific α2-agonist than clonidine, with minor effects on α1-sreceptors. Dexmedetomidine induces a unique sedation pattern, considerably different from all other sedation agents. The patients sedated with this drug easily establish contact, respond to verbal stimuli, communicate and cooperate with ICU personnel, and after establishing contact, they accomplish well at attention tests (5). The recommended sedation depth ranges from 0 to -3 on the RASS scale (mild to moderate sedation) (Table 1). Experimental and clinical studies have indicated that dexmedetomine-induced sedation resembles natural non-REM sleep (6).

Table 1. Richmond agitation-sedation scale

Richmond agitation-sedation scale (RASS)		
+4	Combative	Violent, danger for staff
+3	Very agitated	Pulls or removes tubes and catheters; aggressive
+2	Agitated	Frequent unpurposed movements; intolerant for ventilator
+1	Restless	Anxious, but without aggressive or violent movements
0	Alert and calm	
-1	Drowsy	Not fully alert; having sustained awakening (eye opening/eye contact > 10 s)
-2	Light sedation	Being briefly awake (with eye contact to voice <10 s)
-3	Moderate sedation	Movement and eye opening to voice (without eye contact)
-4	Deep sedation	No response to voice, but movement to physical stimulation
-5	Unarousable	No response to voice or physical stimulation

The usual dosage range is 0.2-0.7 mcg/kg/h, but the safety of higher doses (1.0-1.5 mcg/kg/h) has been demonstrated as well, with careful titration until the desired effect has been achieved. Sedation usually occurs after 15 minutes since the infusion is started, and the maximum effect should be expected within an hour.

Pharmacodynamic properties

Dexmedetomidine is a selective α2-receptor agonist with a broad range of pharmacological actions. It exerts a sympatholytic effect by reducing noradrenaline release in sympathetic nerve endings. Sedative action of dexmedetomidine is mediated by neuronal transmission inhibition in the locus coeruleus in the brain stem, as an important center for the maintenance and modulation of awakeness and attention. Dexmedetomidine has an analgesic effect and also provides the use of smaller amounts of anesthetics/analgesics. Its cardiovascular effects depend on the dosage, and at slower infusion rates the effects on the central nervous system tend to predominate, lowering heart rate frequency and reducing blood pressure. With higher doses, peripheral vasoconstrictive effects predominate, leading to increased systemic vascular resistance and blood pressure, additionally increasing the bradycardic effect. Dexmedetomidine is virtually without depressive effects on the respiratory system when used as a monotherapy in healthy examinees (7).

In postoperative patients in ICUs, who were earlier intubated and sedated with midazolam or propofol, dexmedetomidine significantly reduces the need for additional sedatives (midazolam or propofol) and opioids during sedation in the period up to 24 hours. Most patients receiving dexmedetomidine do not require additional sedative agents. The patients can be successfully extubated without interrupting dexmedetomidine infusion. The studies of individuals who have not been in ICUs confirmed that dexmedetomidine could be safely administered

in patients without endotracheal intubation, provided that they are under appropriate medical surveillance. Dexmedetomidine has shown results similar to midazolam within the targeted sedation extent in mostly nonsurgical patients requiring prolonged mild to moderate sedation (RASS from 0 to -3) in ICUs up to 14 days, with shortened duration of mechanical ventilation compared to midazolam and shortened time to extubation compared to midazolam and propofol. Compared to both propofol and midazolam, the patients are easier to wake up and they are more cooperative and better to communicate with, regardless of the presence of pain.

There have been studies which measured the severity of delirium using the CAM-ICU scale; delirium was reduced in patients receiving dexmedetomidine in comparison to those receiving midazolam, and delirium-associated side effects were more unlikely to occur with dexmedetomidine compared to propofol (8).

Pharmacokinetic properties

Pharmacokinetic properties of dexmedetomidine have been assessed during a short-term IV administration in healthy volunteers and during a long-term infusion in ICU patients. Dexmedetomidine distribution demonstrated a two-compartment model. In healthy volunteers, it demonstrates a rapid distribution phase with the central estimated distribution half-time ($t1/2\alpha$) of about 6 minutes. The mean estimated half-time final elimination value (t1/2) was 1.9 to 2.5 h (min 1.35; max 3.68 h), and the mean estimated value of the volume of distribution in the dynamic equilibrium (Vss) was approximately 1.16 to 2.16 l/kg (90 to 151 l). The clearance (Cl) had a mean value of 0.46 to 0.73 l/h/kg (35.7 to 51.1 l/h). The mean value of body mass related to these Vss and Cl estimates was 69 kg. The plasmatic pharmacokinetics of dexmedeto-midine is similar in ICU patients after an infusion > 24 h. Dexmedetomidine is bound to plasma proteins in 94%. Binding to plasma proteins is constant in the concentration range from 0.85 to 85 ng/ml. Dexmedetomidine binds to serum albumins and alpha-1-acid glycoprotein, with serum albumin being the principal binding protein for dexmedetomidine in the plasma (9).

Biotransformation and elimination

Dexmedetomidine is transformed by hepatic metabolism. There are three types of initial metabolic reactions: N-glucuronidation, N-methylation and cytochrome P450 catalyzed oxidation. Two most prevalent dexmedetomidine metabolites are two N-glucuronide isomers. By way of cytochrome P450 catalyzation two less prevalent metabolites are created. After IV administration of radiolabeled dexmedetomidine, 95% of radioactivity has been found in the urine after nine days, and 4% in the feces, supporting the notion that elimination occurs via the kidney (9).

There were no significant pharmacokinetic differences related to age and gender. Compared to the healthy, binding of dexmedetomidine to plasma proteins is reduced in individuals with hepatic function disorders, necessitating dosage adjustments in such patients. Dosage modification is required as well in patients with kidney failure, since the main route of dexmedetomidine elimination is by renal excretion.

Therapeutic indications

In ICUs, dexmedetomidine is used to sedate adult patients requiring the degree of sedation in which the patient can be woken up by verbal stimulation (RASS values 0 to -3). In those in whom an appropriate degree of sedation cannot be achieved by dexmedetomidine at the maximum dose, another sedation agent should be administered. There have been no clear data about the use of this drug in the periods longer than 14 days.

During dexmedetomidine administration, continuous hemodynamic monitoring is required in all patients. In non-intubated patients, respiratory monitoring is necessary since there is a risk of respiratory depression and, in some cases, apnea.

There have been studies suggesting that bradycardia occurred in relatively healthy non-ICU examinees who received dexmedetomidine. The symptoms receded after leg lifting and anticholinergic therapy such as atropine or glycopyrrolate. In isolated cases, in patients with pre-existing bradycardia, it developed up to the asystolica level. Hypertension was associated with the use of a loading dose, and that reaction could be mitigated by avoiding administering loading doses or by reduced infusion rates or the loading dose level (10).

In the data from clinical studies and data collected after the drug has been made commercially available, there have been several instances of dexmedetomidine overdosing. In these cases, the highest recorded dexmedetomidine infusion rate was up to 60 μg/kg/h for 36 minutes and 30 μg/kg/h for 15 minutes in a 20 months old child and in an adult. The most common reported side effects associated with overdosing in these cases involved bradycardia, hypotension, oversedation, drowsiness and cardiac arrest. In the cases of overdosing with clinical symptoms, dexmedetomidine infusion should be slowed down or stopped. With higher concentrations, hypertension can be more severe than hypotension (11). In clinical studies, bradycardia tended to resolve spontaneously and slowly or it responded to atropine and glycopyrrolate use. Reanimation was necessary in isolated cases of severe overdosing resulting in cardiac arrest.

Since dexmedetomidine must not be used in leading or bolus doses, a physician should be ready to use some other sedative against acute agitation, especially in the first several hours of treatment. Dexmedetomidine should not be used as an induction for intubation or for sedation with the use of muscle relaxants (12).

Dexmedetomidine does not have anticonvulsive effects as some other sedatives and it will not prevent epileptic activity. Caution should be exercised when combining dexmedetomidine with other drugs with sedative or cardiovascular effects, since synergistic action may occur. Dexmedetomidine re-

duces heart frequency and blood pressure by its sympatholytic action, but at higher concentrations, it causes peripheral vasoconstriction leading to hypertension (13). Normally, dexmedetomidine does not produce deep sedation and patients are easily woken up. Dexmedetomidine is therefore unsuitable for patients requiring deep sedation or those with a very unstable cardiovascular system (14).

The use of dexmedetomidine together with anesthetics, sedatives, hypnotics and opioids will probably boost up its action, including sedative, anesthetic and cardiorespiratory effects. Some studies have confirmed increased effects for isoflurane, propofol, alfentanil and midazolam (15). Due to possible pharmacodynamic interactions when anesthetics, sedatives, hypnotics and opioids are combined with dexmedetomidine, it is necessary to reduce their or the dose of dexmedetomidine.

Conclusion

In ICUs, critically ill patients are usually exposed to different therapeutic and diagnostic interventions and environmental stress. Sedatives and analgesics are among the most frequently used drugs aimed to improve the comfort and tolerability of various procedures in ICUs. It has been demonstrated that inadequately treated pain and agitation, and also oversedation, are associated with increased morbidity and mortality rates. Routine monitoring, using reliable scales, enables early detection of agitation and pain, avoiding thus oversedation and severe consequences of delirium. The latest guidelines have advised the use of non-benzodiazepine sedation with dexmedetomidine whenever possible with an aim to improve the outcome in mechanically ventilated critically ill patients. Dexmedetomidine produces a unique sedation pattern, markedly different in comparison to all other sedative drugs. The patients sedated with this drug easily establish contact, respond to verbal stimulation, communicate and cooperate with ICU staff, and after the contact is established they achieve good results at attention tests.

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Pregledni rad **UDC: 615.214.24:616-083**
doi:10.5633/amm.2022.0407

UPOTREBA DEKSMEDETOMIDINA U JEDINICI INTENZIVNOG LEČENJA

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Kritična bolest koja zahteva intenzivan tretman predstavlja veoma stresan događaj. Faktori koji prethode prijemu u jedinicu intenzivne nege su životno urožavajuća stanja, trauma ili veoma složena operacija, koji sami po sebi izazivaju snažnu fiziološku reakciju. Sedativi i analgetici su među lekovima koji se najčešće koriste u intenzivnoj nezi. Njihova upotreba ima za cilj povećanje udobnosti, smanjenje odgovora na stres i olakšavanje dijagnostičkih i terapijskih procedura. Potvrđeno je da su bol, prekomerna sedacija i delirijum značajni uzročnici stresa kod bolesnika u intenzivnoj nezi i povezani sa povećanim morbiditetom i mortalitetom. Termin „trijada intenzivne nege" opisuje blisku povezanost bola, uznemirenosti i delirijuma, kao i pristup njihovom lečenju. U smernicama iz 2013. i 2018. godine za analgeziju i sedaciju kod kritično bolesnih preporučena je upotreba midazolama, samo za kratkotrajnu sedaciju, lorazepama, za dugotrajnu sedaciju, a propofola za bolesnike kod kojih je planirano povremeno buđenje. U novim verzijama smernica data je prednost sedativima koji nisu benzodiazepin, kao što je deksmedetomidin. Deksmedetomidin proizvodi jedinstveni obrazac sedacije, koji se značajno razlikuje u poređenju sa svim drugim sedativnim lekovima. Bolesnici sedirani ovim lekom lako uspostavljaju kontakt, reaguju na verbalnu stimulaciju, komuniciraju i sarađuju sa osobljem intenzivne nege, a nakon uspostavljanja kontakta, postižu dobre rezultate na testovima pažnje.

Acta Medica Medianae 2022;61(4):48-53.

Ključne reči: *deksmedetomidin, sedacija, jedinica intenzivne nege, delirijum*

Review article

UDC: 616.24-008.4:612.821.7
doi:10.5633/amm.2022.0408

PERSONALIZED TREATMENT OF OBSTRUCTIVE SLEEP APNEA: IS IT STILL A LONG WAY OFF?

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The combination of sleep fragmentation, intermittent hypoxia exposure and circadian rhythm misalignment is crucial to represent multiple obstructive sleep apnea (OSA) clinical scenarios. Treatment of OSA has traditionally been directed to anatomical component treatment, implying the application of continuous positive airway pressure (CPAP) therapy, oral devices, upper airways surgery, weight loss, and positional therapy. These therapeutic approaches may be frustrating, especially in patients who fail to tolerate CPAP therapy, they may require personal engagement and are hardly maintained, or they have variable and hardly predictable efficacy. So, new treatment approaches aiming at specific, treatable, phenotype characteristics of OSA are needed as alternative therapeutic options.

Acta Medica Medianae 2022;61(4):54-62.

Key words: *OSA phenotypes, personalized treatment of OSA*

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Introduction

Obstructive sleep apnea (OSA) is life-threatening disease that causes significant economic burden if untreated, and it is characterized by intermittent hypoxia, sleep fragmentation and sleep deprivation. Benjafield et al. estimate that 936 million people aged 30-69 years may have mild to severe OSA (1). When not treated, OSA is linked with long-term, health-related consequences, but also decreased work productivity and work-related accidents and motor vehicle accidents (2). The results of eighteen-year follow-up of the Wisconsin Sleep Cohort study show that severe OSA is associated with a 3-fold increase in total mortality risk ($p < 0.0008$), in-dependent of age, sex, body mass index (BMI), but after exclusion of patients who reported using a continuous positive airway pressure (CPAP) therapy at night, this association was even higher, 3.8-fold increase in total mortality (3).

The gold standard for diagnosing sleep breathing disorders is polysomnography (PSG) under controlled conditions. It is demanding, time-consuming and expensive test. Although there are many neurophysiological signals obtained during PSG, the treatment decision is greatly based on the apnea-hypopnea index (AHI), which represents number of cessations in breathing – apneas and periods of reduction in airflow – hypopnea longer than 10 seconds per hour of sleep, causing a drop in oxygen saturation (SATO2) or arousal, which shows some limitations. For example, patients in whom respiratory events last longer may have significant hypoxemia with relatively low AHI, and vice versa, shorter events and significantly higher AHI with minimal exposure to hypoxemia and its negative impact. Recent studies have shown that apnea during REM sleep may be significant for the development of insulin resistance and cardiovascular side effects of OSA (4, 5). It is becoming clear that OSA involves a clinical spectrum that greatly surpasses classic manifestations regarding male, obese and sleepy patients (6). Lack of drowsiness does not exclude the presence of significant breathing disturbances in sleep, 25% moderate OSA patients are not sleepy at all. Up to 50% OSA patients are not obese (7, 8).

OSA pathogenesis

Just like heterogeneity in clinical presentation, OSA pathogenesis is diverse too. Certain degree of anatomic impairment (narrow, collapsible)

of the upper airways is of crucial importance. Since the obstruction occurs only during sleep, dynamic, non-anatomical factors (insufficient or reduced pharyngeal dilatator muscle activity during sleep, low respiratory cortical arousals threshold, unstable respiratory control system – high 'loop gain') present in about 70% of OSA patients, play an important role in mediating the presence or absence of OSA (9, 10).

Anatomical causes of OSA are generally heterogeneous ones, with potential multi-level obstruction of the upper airways. Only 25% of patients have one-level obstruction, while 75% have multilevel obstruction sites (11). The structures that may contribute to upper airway narrowing and collapse include soft palate, tongue size and position, epiglottis and lateral pharyngeal walls, pharyngeal dilator muscle, primarily m. genioglossus, hyoid bone position, and upper airways surface tension. The severity of OSA is independently associated with increased expiratory tracheal collapse (12). Obesity is an important risk factor. Pharyngeal fat deposits cause a reduction in pharyngeal volume. Neck circumference has routinely been used as a risk predictor of OSA. Although PSG is a gold standard for OSA diagnosis, it does not provide information on obstruction localization. Several other diagnostic modalities have been demonstrated to usefully supplement physical examination: lateral cephalogram, 3-dimensional computed tomographic scan, drug-induced sleep endoscopy (DISE), dynamic magnetic resonance imaging (cine-MRI). The last two modalities are promising since they evaluate static and dynamic aspects of the upper airways during sleep and in sleep-like state. Restrictions on clinical use include:

1) awake static imaging does not provide an insight into dynamic characteristics of upper airways during sleep;

2) lack of clear imaging protocols; and

3) high costs (13, 14).

The best measure to describe functional anatomy of upper airways during sleep is pharyngeal critical closing pressure (Pcrit) (15). Pcrit values of OSA patients are usually similar to the values of atmospheric pressure, showing that obstruction of their airway is at or near 0 cmH2O during sleep. Pcrit may vary from: -5 to >5 cmH2O. Pcrit value +5 cmH2O or close shows that airways are highly collapsible, while a sub-atmospheric Pcrit indicates relatively stable upper airways, since suction pressure is required for an airway to be closed during sleep. The values of sub-atmospheric range in this group (0 to -5 cmH2O) are important because of overlapping in Pcrit between healthy and affected individuals. Near 20% of OSA patients have Pcrit like healthy population. This group of patients, in whom the pathogenesis is dominated by a mild anatomical predisposition in combination with one or more non-anatomical causes of OSA, is more likely to benefit from non-CPAP targeted therapies than those who have high Pcrit (16). Considering that current technique of measuring Pcrit is invasive, technically complex, needs CPAP utilization and pharyngeal pressure catheter, a simpler Pcrit measuring would be of great importance for making decisions on targeted treatment (17). There are some innovative and effective methods for determining the level of anatomical damage in OSA patients, including a method for assessing expiratory flow limits in individuals with chronic obstructive lung disease using an existing methodology; Genta et al. have revealed that analyzing the shape of the inspiratory flow curve during constrained airflow during sleep, as well as the degree of negative effort dependence, might reveal the location of obstruction; simple quantifying of peak flow during PSG showed association with active Pcrit (a measure including upper airways collapsibility and neuromuscular compensation); additionally, during routine CPAP titration, the prescribed level of CPAP pressure is linked to Pcrit and may be useful in discriminating between patients with mild and extremely collapsible airways: therapeutic level of CPAP ≤ 8.0 cmH2O showed sensitivity of 89% and specificity of 84% for detecting mild collapsibility, but after independent validation the specificity was 91%, but sensitivity was reduced to 75% (18-21).

Traditional therapeutic approach

Treatment of OSA has traditionally been directed to anatomical component treatment, implying the application of CPAP therapy, oral devices, upper airways surgery, weight loss, and positional therapy. Currently, CPAP therapy is the gold standard, especially in severe OSA cases. The mechanism of action provides pneumatic splint to maintain patency of upper airways. CPAP therapy also stabilizes the upper airway by increasing expiratory reserve lung volume. Advancement in CPAP technology and optimal choice of masks may improve comfort and tolerance, but poor adherence rate is often high (> 50% in some countries). About 5 million, 85% of diagnosed OSA patients in the United States of America, receive the positive airway pressure (PAP) treatment, whether it is CPAP, AutoPAP or Bilevel PAP, but only 3 million, 60%, will adhere to treatment in the long terms. Although daytime sleepiness was minimal, a minimum average use of CPAP therapy was only 3.3 hours per night, as reported by a large randomized trial on CPAP therapy. This level of adherence has no cardiovascular benefit. Long-term CPAP therapy adherence is significantly associated with younger age, female gender and increased sleepiness, but not with OSA severity (22-24). Oral devices are used as a backup to CPAP therapy or as a first-line treatment if CPAP therapy fails. The overall response rate (AHI 5) varies between 21% and 71%. About 25% of individuals with severe OSA are included in this response rate. The partial response rate (> 50% reduction in AHI) varies between 6% and 63% (25). Clinical success of surgical treatment is defined as a reduction in AHI from greater than 50% to less than 20%, with rates ranging from 5% to 78%. Changes in pharyngeal morphology and upper airway muscle activity during waking or sleeping periods may be major determinants of therapy response rate (26, 27). Weight loss reduces the severity of OSA, although in different range, by dietary modifications (3%-18% body weight reduction reduces AHI from 3% to 62%),

and after bariatric surgery (12%-37% body weight reduction reduces AHI from 48%-90%). Variability is also influenced by baseline BMI and adipose tissue distribution (28).

These treatments can be annoying, especially for patients who are unable to tolerate CPAP therapy; they may necessitate personal engagement and are difficult to sustain (weight loss); or they may have variable and unpredictable efficacy (oral devices, upper airways surgery, positional therapy). As a result, instead of 'one-size-fits-all,' novel treatment approaches aimed at specific, curable phenotypic traits are required as alternative therapeutic options (29).

New therapeutic options

The pharyngeal muscles are crucial for maintaining the patency of the upper airways. They receive complex signals synchronized with inspiration from respiratory tract neurons to strengthen and dilate airways and prevent inspiratory collapse. They also get reflex signals from pressure-sensitive mechanoreceptors in the airways and chemoreceptors that detect CO2 or O2 changes. An major component to OSA pathogenesis is a reduction or loss of central and reflex stimulation to the upper airways during sleep (30). The relation between upper airway muscles activation and stimuli (measured via Pcrit) is known as muscle responsiveness (31). Some OSA patients have a high threshold to stimuli during sleep that cannot be reached without waking up, while others can recover airflow during sleep through pharyngeal muscle activation without waking up. High upper airways muscles responsiveness may be a protection against OSA development despite anatomical predisposition. The upper airway contains over 20 muscles. They help in speech, swallowing, and chewing, in addition to maintaining airway patency. In the pathophysiology of OSA, m. genioglossus is the largest and most studied pharyngeal dilator muscle. M. genioglossus response to airway narrowing during sleep is low in about 30% of OSA patients (32, 33).

Hypoglossal nerve stimulation with an implanted neurotransmitter is a method for activating upper airway muscles while sleeping. Electrical stimulation has been tested to treat OSA since the 1980s, with a variety of techniques, invasive ones, as described in STAR trial, and non-invasive, as studied in TESLA trial. Improvements in subjective drowsiness, as well as significant reductions in AHI and oxygen desaturation index, have been reported in recent research (ODI). It is difficult to select potential patients who could benefit from this treatment, what can be related to individual Pcrit (anatomical pharyngeal configuration and the site of the collapse). Endoscopy was employed in the STAR study as a screening tool to rule out individuals with concentric upper airway collapse, which could improve therapeutic response rates. However, one-third of patients were classified as those without improvement. This treatment strategy may play a role in patients with moderate to severe OSA in whom CPAP therapy failed, with BMI < 32 kg/m^2, and without significant collapse during DISE (34-39).

The results of nine studies, included 120 adult patients with OSA, showed that oropharyngeal exercises reduced AHI by about 50% and increased lowest SATO2 > 2.5%, improved subjective sleepiness about 45% (> 6 points reduction according to the Epworth Sleepiness Scale), and reduced snoring (40).

There is no approved pharmacotherapy for OSA yet, but various attempts have been made and are still being made to find one (41).

There have been recent advancements in understanding pharmacological measures for improving upper airway muscles. The introduction of potassium channel blockers into the pigs' nostrils activated mechano-receptors, which increased pharyngeal muscle activity and reduced upper airway collapsibility. In healthy adults without OSA, a recent research using 10mg 4-aminopyridine orally, a very strong potassium channel blocker, only slightly increased m. genioglossus activity in the REM phase but not in non-REM sleep (42, 43). Desipramine, a tricyclic antidepressant with strong noradrenergic, mild serotonergic, and mild antimuscarinic effects, reduces collapsibility of upper airways and OSA severity, preventing sleep-induced reduction of m. genioglossus activity, especially in patients with minimal muscle responsiveness (44). It is interesting that hypnotic zolpidem has the potential of increasing pharyngeal muscle responsiveness during airway narrowing without compromising other important OSA causes (45). In REM sleep, muscarinic receptors antagonists exhibited a particularly strong restorative effect on m. genioglossus activity. In a preliminary proof-of-concept research, these findings were recently applied to humans. The m. genioglossus reactivity to negative esophageal pressure swings was increased near threefold with atomoxetine combined with oxybutynin, and the AHI was reduced by 63% in both REM and NREM sleep, and oxygen saturation parameters improved as well.

Since the majority of obstructive events are related to cortical arousal, respiratory cortical arousals were considered crucial for airway reopening after an obstructive event in OSA patients. About 20% of obstructive events, however, end without respiratory cortical arousal, and another 20% occur after the upper airway is already reopened and airflow is established. So, airway reopening may be restored without arousal (46). When an increase in negative intrathoracic pressure reaches a particular degree of respiratory arousal threshold, respiratory cortical arousals from sleep during an obstructive event occur (47). The gold standard for determining the respiratory cortical arousals threshold is to use a PSG and an epiglottic or esophageal pressure catheter. The negative pressure just before cortical arousal is the respiratory cortical arousal threshold. Patients with OSA who require a considerable change in intrathoracic pressure to trigger respiratory cortical arousal (high respiratory arousal threshold 25 cmH2O) frequently have extended respiratory events, particularly if they also have poor upper airway muscle response (47). On the other hand, 30%-50% of OSA patients (> 85% of non-obese

patients) wake up too easily to modest changes in intrathoracic pressure (between 0 and -15 cm H2O), which may impede proper responsiveness of upper airway muscles as compensation mechanisms to stabilize breathing (48, 49). Frequent arousals cause sleep fragmentation, change sleep architecture by preventing deeper stages of sleep and enhancing sleep instability. So, strategy in reducing respiratory cortical arousals in these patients may stabilize breathing during sleep. Having in mind the aforementioned facts, the potential therapeutic role of hypnotic drugs in treating OSA patients with a low respiratory cortical arousals threshold phenotype has been a field of interest in a lot of studies. An increase in respiratory cortical arousals threshold must be without decreasing pharyngeal muscles activity. Simultaneously, hypnotic use in patients with a high respiratory cortical arousal threshold may prolong respiratory episodes and aggravate hypoxemia, particularly in obese individuals with advanced illness. Standard doses of trazodone (100 mg), zopiclone (7.5 mg) and eszopiclone (3 mg) raise the respiratory cortical arousal threshold and may lower AHI by 25% to 50% without affecting pharyngeal muscle response or increasing hypoxemia levels. However, high doses, especially in patients with severe OSA may prolog obstructive events and worsen hypoxemia. Paradoxically, zolpidem increases m. genioglossus activity almost three-fold both in healthy and in OSA patients (50, 51). These findings highlight hypnotics' therapeutic potential in carefully selected patients (those with a low respiratory cortical arousal threshold and SATO2 > 70%) (52, 53). The current method for determining respiratory cortical arousal threshold is inconvenient for routine clinical usage since it is time-consuming, expensive, and unpleasant (requires an airway pressure catheter). Based on three variables obtained by standard PSG (AHI, lowest SATO2, and apnea/hypopnea ratio), Edwards et al. developed a simple method for estimating respiratory cortical arousal threshold with high sensitivity and specificity that could be used in clinical practice after additional tests were completed (49).

'Loop gain' describes the sensitivity of the negative feedback loop that controls ventilation, which is utilized to keep blood gas tension levels within determined limits (41). Namely, during sleep, PaCO2 precisely regulates ventilation by chemoreceptors afferent feedback information. The ratio between ventilator response and ventilator disturbance is known as 'loop gain' in respiratory physiology. It consists of three components:

1) tissue (tissue, blood and lungs with CO2)

2) release in circulation (time needed for a change in CO2 concentration to mix with existing blood, to come and be detected by chemoreceptors), and

3) sensitivity to CO2 concentration change (chemosensitivity).

Any medical condition that alters one or more of the aforementioned components (for example, heart failure) alters 'loop gain'. Respiratory control can also be affected by intermittent hypoxia (54, 55). Elevated 'loop gain' shows unstable ventilation control. People with high 'loop gain' have exaggerating ventilator response to minimal changes in CO2 concentration. This can lead to hypocapnia and respiratory drive reduction, and may result in repetitive upper airway collapse. About 30% of OSA patients have high 'loop gain' (less than -5) which shows an increase in ventilation for over 5 l/minute in response to reduction in minute ventilation of 1 l/minute. More negative number reflects higher 'loop gain'. High 'loop gain' in combination with even a moderate collapsibility of upper airways may initiate OSA pathogenesis. On the other hand, hypoventilation commonly occurs during sleep in those with extremely low 'loop gain', such as those with obese hypoventilation syndrome. 'Loop gain' can be measured utilizing transitory pressure drops in CPAP during sleep until obstructive events occur, then quick reintroduction of CPAP with a breathing mask and pneumotachograph to determine 'loop gain' (33, 56-58). This method requires well trained staff and complicated data analysis. Terrill et al developed a method for evaluating 'loop gain' that employs the nasal pressure data from a typical PSG (59).

Because oxygen possesses ventilatory stabilizing qualities, primarily due to a decrease in peripheral chemosensitivity, it might theoretically be a therapy option for OSA patients with higher-than-normal loop gain (41). In OSA patients with high loop gain, oxygen therapy reduces loop gain and lowers AHI by nearly half (60). However, all of the studies that have looked into this issue so far have had varying initial hypoxia and normoxia levels, as well as an emphasis on monitoring various outcomes with varying quantities of supplemental oxygen for a maximum of three months. Patients with OSA who have intermittent hypoxia and have a SATO2 reduction of 94% to 85% differ significantly from those who have a SATO2 reduction of 95% to 91%. Trials aimed at determining the phenotypes and endotypes of OSA have concentrated on a small number of patients selected among thousands of OSA patients. The suppression of hypoxic respiratory stimulation, with simultaneous increase in hypercapnia and acidosis development, has not been fully understood yet, even without associated respiratory pathologies. Maybe in future, with the development of better and more simple methods for identifying different OSA phenotypes, application of oxygen therapy alone, or in combination with other therapeutic approaches, may be justifiable in appropriately selected patients (increased loop gain, or patients with higher nocturnal intermittent hypoxia), but not in others. Currently, we still have a long way to go before its routine application in the treatment of these patients (61, 62). Acetazolamide (500 mg twice daily for one week) is a carbonic anhydrase inhibitor that reduces loop gain in OSA patients by roughly 40% (63). Zonisamide, also having carbonic anhydrase inhibitory features, reduces AHI in obese patients with severe OSA (64). Unwanted side-effects reported by patients (dizziness, taste changes, dry mouth) may limit long-term tolerance for carbonic anhydrase inhibitors. Aminophylline, theophylline, and caffeine are xanthines that improve diaphragm contractility by antagonizing adenosine in the central nervous system. While xanthines have shown to be useful in reducing central

apneas in premature infants and patients with heart failure and periodic breathing, they have had mixed outcomes in individuals with OSA. Donepezil is a reversible acetylcholinesterase inhibitor that boosts cholinergic transmission to muscarinic and nicotinic receptors. Donepezil was first tested on OSA severity in a group of 11 patients with Alzheimer's disease and compared to placebo (N = 12) in a parallel arm trial by Moraes et al., based on the involvement of cholinergic systems in ventilatory control during sleep and evidence that reduced thalamic cholinergic activity was associated with OSA severity in patients with multisystem atrophy. In this population, Donepezil 10 mg resulted in a 50% reduction in AHI after three months of treatment. Sukys-Claudino et al tested the same medicine in 11 OSA patients without Alzheimer's disease for one month and found a 23% reduction in AHI from baseline and a 39% reduction compared to placebo. Oxygen saturation and drowsiness improved as well, however sleep efficiency was lowered on the medicine (41).

Recent studies have highlighted a potential for combining different therapeutic approaches as an effective alternative to single therapeutic options in many OSA patients. For example, by combining two therapeutic options targeting anatomical features, such as positional therapy and hearing aids, AHI reduces by about 75% in comparison to a reduction of about 50% when they are used alone. A targeted phenotypic approach combined with non-CPAP therapy (including hypocaloric diet) and pharmacological agents (trazodone and/or acetazolamide) decreases symptoms by 65%. In a small group of patients, combining oxygen therapy to diminish loop gain with a hypnotic to raise cortical arousals threshold reduces AHI by 95% (65-67).

Pcrit, respiratory cortical arousals threshold, loop gain and muscle responsiveness−PALM approach of targeted therapy has been developed according to the phenotype concepts as an addition to already present clinical determinants (symptoms, AHI, comorbidities) to facilitate overall personalized approach for OSA treatment. Briefly, having in mind that anatomical component is a key force in OSA, it is highlighted that this feature is the most important determinant in treatment decision. OSA patients with only mild anatomical impairment (Pcrit < -2 cmH2O), about 19%, have, as a rule, significant impairment of a non-anatomical component. It is estimated that the application of therapies targeting non-anatomical causes will solve OSA in these patients. Moderate anatomical impairment (Pcrit -2 to 2 cmH2O) affects about 58% of OSA patients. Non-anatomical mechanisms are present in approximately two-thirds of these patients. These patients are expected to benefit the most from a combination of targeted anatomical and non-anatomical interventions (e.g. oral devices and oxygen therapy). The remaining third of patients with moderate anatomical impairment who do not have a significant contribution from non-anatomical causes will most likely require one or more anatomical problem-specific therapies (positional therapy, CPAP therapy, surgical interventions). Twenty-three percent of OSA patients have severe anatomical impairment (Pcrit > 2 cmH2O), so they require a therapy targeted at its treatment (CPAP therapy) (68).

A European Respiratory Association research seminar titled 'Defining harmful effects of sleep disturbances and disorders' was held in Dublin in 2019. It was the first step in promoting scientific cooperation and building a critical mass of European research consortiums. The ability to identify illness risk at an individual level, understanding biological mechanisms responsible for disease development and comorbidity onset, and early start in applying individualized therapies are all important aspects of the future of medicine, according to the seminar's conclusion. Prerequisites for achieving these personalized medicine goals in OSA include:

1. definition of relevant phenotypes using real-world data from well-selected cohorts;
2. the combination of sleep fragmentation, intermittent hypoxia exposure and circadian rhythm misalignment is crucial to represent multiple OSA clinical scenarios;
3. cell cultures of animal and human models in intermittent exposure to hypoxia should be technically improved by including hypercapnia as an associated stimulus;
4. activities of the disease should be viewed through the prism of the onset and progression of comorbidities in OSA patients, with initiation of early personalized interventions;
5. application of innovation in clinical research methods to decrease costs and increase productivity;
6. artificial intelligence will be crucial in monitoring dynamics and heterogeneity of OSA longitudinal studies. This will also be the case with new techniques for identifying abnormal respiratory events during sleep or new methods for analyzing sleep electroencephalogram (69).

Conclusion

Recent identification of various OSA phenotypes has created a basis for applying targeted therapy. Development of simplified approaches to identify certain phenotypes is crucial in well-selected groups of patients. Further well-designed studies are needed to define risks/benefits of such a treatment approach.

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Pregledni rad **UDC: 616.24-008.4:612.821.7**
doi:10.5633/amm.2022.0408

PERSONALIZOVANA TERAPIJA OPSTRUKTIVNE SLEEP APNEJE – KOLIKO SMO DALEKO?

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Kombinacija fragmentacije sna, povremene izloženosti hipoksiji i neusklađenosti cirkardijalnog ritma presudna je za ispoljavanje višestrukih kliničkih scenarija opstruktivne sleep apneje (OSA). Lečenje OSA tradicionalno je usmereno na lečenje anatomske komponente i podrazumeva primenu terapije kontinuiranim pozitivnim pritiskom (CPAP) tokom sna, primenu oralnih aparata, operacije gornjih disajnih puteva, gubitak težine i pozicionu terapiju. Ovakav pristup lečenju može biti frustrirajući, posebno za bolesnike, koji ne tolerišu CPAP terapiju, zahteva veliko lično angažovanje i teško je održiv ili ima promenljivu i teško predvidljivu efikasnost. Dakle, novi terapijski pristupi, koji ciljaju specifične, lečive, fenotipske karakteristike OSA, neophodni su kao alternativa.

Acta Medica Medianae 2022;61(4):54-62.

Ključne reči: *OSA fenotipovi, personalizovana terapija OSA-e*

Review article **UDC: 616-089.163-083+616-005.6-085:615.27.53**
doi:10.5633/amm.2022.0409

PREOPERATIVE MANAGEMENT OF PATIENTS ON CHRONIC ANTITHROMBOTIC THERAPY WHO REQUIRE ELECTIVE NON-CARDIAC SURGERY

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Preoperative management of antithrombotic therapy (antiplatelet and anticoagulant therapy) is challenging since discontinuation of therapy carries a risk of the thromboembolic event and surgery carries a risk of bleeding. An optimal balance between thromboembolic and bleeding risk must be reached and the decision whether to stop antithrombotic therapy or not be made. Each patient requires an individual assessment. That means estimating bleeding and thromboembolic risk for each patient. Bleeding risk is based on patient-related risk factors and risk associated with the surgical procedure. Thromboembolic risk is more complex to calculate. If the decision is to stop antithrombotic therapy, the next question is how long before the surgery it should be stopped and whether the bridging therapy is required.

Acta Medica Medianae 2022;61(4):63-72.

Key words: *antithrombotic therapy, thromboembolic risk, bleeding risk*

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Introduction

Antithrombotic (AT) therapy is used to minimize the incidence of arterial and/or venous thrombosis in patients at increased risk. At increased risk are patients with atrial fibrillation (AF), mechanical heart valves (MHV), recent arterial or venous thromboembolism (VTE), patients with implanted stents (1). The most widely used AT drugs are antiplatelet (AP) drugs like acetylsalicylic acid (ASA) and P2Y12 inhibitors and anticoagulant (AC) drugs like vitamin K antagonists (VKA) and direct oral anticoagulants (DOAC) (2). A lot of patients on AT therapy will need surgery and preoperative management is very complex. If therapy is stopped, the risk of thrombosis during and after surgery is increased. Not stopping the therapy increases the risk of bleeding. The balance between thromboembolic (TE) risk and bleeding (BL) risk has to be made. In order to make this balance we need to estimate the individual's risk of thrombosis, the individual's risk of bleeding and the risk of bleeding that surgery carries. If the decision is to stop AT therapy, the next question is the timing of interruption and using of bridging therapy (3-5). We will answer these questions in our paper.

Thromboembolic risk in patients on anticoagulant therapy

There are three main conditions associated with increased risk of thromboembolism: AF, MHV and recent VTE.

Atrial fibrillation

The most common reason of preoperative AC use is AF. Six million patients with AF require surgery each year (6). The TE risk in these patients is estimated by CHA2DS2VASc score (Table 1). This score is based on the presence of congestive heart failure, hypertension, patient's age, diabetes mellitus, history of stroke/TIA, vascular disease and sex. The highest score is 9 points and a total score of 2-3 points means low TE risk (< 5%), 4-5 points moderate TE risk (5-10%) and a score over 6 points high TE risk (< 10%) (Table 2) (7-9).

Mechanical heart valves

TE risk in patients with MHV without AC therapy is 8-22% (9). The use of warfarin reduces this

risk by 80% (10). The INR in patients with aortic MHV without other TE risk factors should be 2.5, while in those with associated TE risk factors or mitral MHV the INR should be 3.0. Additional risk factors include AF, ejection fraction (EF) lower than 40%, older age, hypercoagulable state and previous TE. The location, type and number of MHV are important indicator of TE risk. The highest risk is with multiple MHV, followed by mitral MHV and lowest with aortic MHV (Table 2) (11).

Recent thromboembolism

The greatest risk is immediately after TE event and decreases over time. Therefore, surgery should be delayed for three months, if possible. Recurrence of VTE is greatest in 3-4 weeks after initial event and decreases in the next three months. Without AC therapy the probability of recurrent VTE is 50%. Warfarin therapy reduces this risk to 8-10% after one month of therapy and to only 4-5% after 3 months (12). If urgent surgery is needed, bridging therapy can be used to reduce the TE risk. The greatest risk is in patients with VTE or pulmonary thromboembolism (PTE) in last 3 months and in those with VTE associated with congenital thrombophilia. A TE event that occurred more than a year ago means low TE risk and the presence of malignancy moderate (Table2) (13, 14).

Table 1. CHA2DS2VAS score

Letter	Risk factor	Score
C	Congestive heart failure (EF < 40%)	1
H	Hypertension	1
A2	Age ≥ 75	2
D	Diabetes mellitus	1
S2	Stroke/TIA	2
V	Vascular disease	1
A	Age 65-74	1
S	Sex category (female)	1
	Maximum score	**9**

Table 2. Thromboembolic risk by anticoagulation indication

Risk	Atrial fibrillation	Mechanical valves	Venous thromboembolism (VTE)
Low (annual risk of VTE < 5%)	CHA2DS2VASc score 2-3	Bileaflet aortic valve without additional risk factors	VTE more than 12 months ago without additional risk factors
Moderate (annual risk of VTE 5-10%)	CHA2DS2VASc score 4-5 Stroke, TIA more than 3 months ago	Bileaflet aortic MHV and one or more additional risk factors: age>75, congestive heart failure, AF, previous stroke/TIA	VTE within 3-12 months Recurrent VTE active cancer non severe thrombophilia
High (annual risk of VTE > 10%	CHA2DS2VASc score > 6 Stroke, TIA in the last 3 months	Mitral Aortic (cage ball or tilting disk) Stroke, TIA in the last 6 months	VTE in the last 3 months severe thrombophilia

Thromboembolic risk in patients on antiplatelet therapy

The most common indication for AP therapy is the prevention of development and recurrence of arterial ischaemic events, including coronary artery disease (CAD), cerebrovascular events and peripheral arterial disease (15). Patients with CAD often undergo percutaneous coronary intervention (PCI) with stent implantation.

In North America, 5% of patients undergo non-cardiac intervention within 1 year of stenting (16). The period between the TE event and the surgery is the most substantial in estimating TE risk in patients on dual antiplatelet therapy (DAPT). The studies show that the greatest risk of TE event is present in patients who have surgery within 6 months of stent implantation (17). The next important factor is the type of stent. PCI with first generation Drug Eluting Stents (DES) has a higher incidence of thrombosis than those with Bare Metal Stents (BMS). Second-generation DES provide greater safety and lower incidence of thrombosis compared to BMS. Although experience with bio-

resorbable stents is limited, some research has shown an increased incidence of stent thrombosis. It is recommended that DAPT lasts at least 12 months for these stents. Since there is no risk of stent thrombosis, patients undergoing coronary artery bypass grafting (CABG) or non-invasive drug treatment are at lower risk of complications. The next important determinant is whether the disease is stable or not. Individuals with stable coronary artery disease have shown to be at a lower TE risk than those with acute coronary syndrome (ACS). Patient's comorbidities must also be considered. Patients who have had complex PCI also have a higher risk of thrombosis. Intervention in people who have had a stroke is also a risk factor, especially in the first 30 days after a stroke. Stent thrombosis in peripheral arterial disease is most common in the first month. The risk is highest in DES or stents in chronic occlusion. In patients with stent implantation in the lower extremities, the use of DAPT for at least 1 month has been suggested in the guidelines (Table 3) (14, 18-20).

Table 3. Thromboembolic risk by antiplatelet indication

Indication for antiplatelet therapy					
Risk	**Time on therapy (months)**	**ACS**	**Stable coronary artery disease**	**Cerebro-vascular disease**	**Peripheral artery disease**
High	< 3	Medical treatment	PCI + BMS/DES/DEB or CABG	Stroke, carotid stent placement	Acute TE on peripheral blood vessels + revascularisation or chronic occlusion
	< 6	PCI + BMS/DES/DEB or CABG	PCI + BMS/DES/DEB or CABG + risk factors		
	< 12	PCI + BMS/DES/DEB or CABG + risk factors PCI + first generation DES BVS	PCI + first generation DES BVS		
Moderate	3-6	Medical treatment	PCI + BMS/DES/DEB or CABG	Stroke, carotid stent placement	Acute TE on periphery blood vessels + revascularisation or chronic occlusion
	6-12	PCI + BMS/DES/DEB or CABG	PCI + BMS/DES/DEB DEB or CABG + risk factors		
	> 12	PCI + BMS/DES/DEB or CABG + risk factors PCI + first generation DES BVS	PCI + first generation DES BVS		
Low	> 6	Medical treatment	PCI + BMS/DES/DEB or CABG	Stroke, carotid stent placement	Acute TE on periphery blood vessels + revascularisation or chronic occlusion
	> 12	PCI + BMS/DES/DEB or CABG	PCI + BMS/DES/ or CABG + risk factors		

Bleeding risk

The most important factors in estimating the BL risk include the type of surgery and the clinical characteristics of the patient. These characteristics are determined by using the HAS-BLED bleeding risk score (H–hypertension, A–abnormal renal/liver function, S–stroke, B–bleeding tendency, L–labile INR, E–elderly, D–drugs or alcohol use) (Table 4). The score assigns 1 point for each of the risk factors and a HAS-BLED score greater than 3 points indicates an increased BL risk. The BL risk is divided into low (0-2% two-day risk of bleeding after surgery) and high risk (2-4% two-day risk of bleeding after surgery) (21). Examples of high BL risk interventions include major intraabdominal, major orthopedic, cardiac surgery, lung resection surgery, extensive cancer surgery, major urologic and vascular surgery. Examples of low BL risk interventions are cataract surgery, dental extraction surgery, skin biopsy, gastroscopy or colonoscopy without biopsy, carpal tunnel repair. Surgical interventions involving neuraxial, intracranial and cardiac sites are of special interest because of the anatomical localization of the source of hemorrhage (Table 5) (22).

Table 4. HAS-BLED score

Risk factor	Score
Hypertension	1
Abnormal renal/liver function	1 or 2
Stroke	1
Bleeding tendency	1
Labile INR	1
Elderly (age > 65)	1
Drugs (like aspirin, NSAIL), alcohol	1 or 2
Maximum score	9

Table 5. Bleeding risk according to procedure

High bleeding risk interventions	Low bleeding risk interventions
Major intracranial or neuraxial surgery	Gastrointestinal procedures (colonoscopy, gastroscopy, sigmoidoscopy, ERPC)
Major thoracic surgery (lobectomy, pneumonectomy, esophagectomy	Cardiac procedures (PCI, internal cardiac defibrillator implantation, coronary artery angiography
Major cardiac surgery	Dental procedures
Major vascular surgery (aortic aneurysm repair, aortobifemoral bypass, popliteal bypass)	Skin interventions (skin biopsy)
Major abdominal/pelvic surgery (hepatobiliary cancer resection, pancreatic cancer, colorectal and gastric cancer resection, bladder cancer resection, endometrial and ovarian cancer resection)	Eye interventions (cataract)
Major orthopedic surgery (hip arthroplasty, knee arthroplasty, shoulder arthroplasty)	
Other major cancer or reconstructive surgery	
Any surgery requiring neuraxial anesthesia	

Interrupt anticoagulant therapy or not?

An optimal balance between TE and BL risk must be made. Clinical assessment is imperative. Individuals at high BL risk will benefit from discontinuation of AC therapy. On the contrary, the patients at high TE risk require bridging and a period without anticoagulants as short as possible. Those scheduled for low BL risk surgery very often do not have to stop AC therapy. Efforts should be made to keep the risk of both bleeding and thrombosis as low

as possible, regardless of the status of AC therapy (Figure 1) (6, 23).

Timing of anticoagulant interruption

Therapy should be discontinued within a time frame sufficient to withdraw the effect of the drug. For some drugs, such as VKA, laboratory tests are a reliable indicator of AC activity. However, for DOACs, such tests are not always available.

Warfarin

Stop 5 days before surgery (last dose on a day minus 6). PT/INR should be checked one day prior to surgery. If the INR is higher than 1.5, give a low dose of vitamin K (1-2 mg) and check the INR on the morning of surgery (23). It is especially important that the INR is within the reference values in high BL risk surgeries or if neuraxial anesthesia is given. The timing of warfarin interruption is based on its half-life (36-42 h) and on the period of time for PT/INR to return to normal (2-3 days to INR fall to 2; 4-6 to become normal). This time may be longer in patients with higher INR values and in the elderly. Half-lives of some other VKA are longer (8-11 h for acenocoumarol, 4-6 days for phenprocoumon, 3 days for fluindione). This discontinuation schedule leads to several days when the AC effect is subtherapeutic, which requires bridging in those with a high or very high risk of TE event. Preoperative bridging is reserved for patients at high TE risk (e.g., recent stroke, MHV, CHA2DS2-VASc score 5, 6, 7, 8) who require discontinuation of AC therapy. The bridging drug is given in a therapeutic dose 3 days before the operation (13, 24, 25).

Direct oral anticoagulant drugs

Due to rapid cessation and rapid onset of action, patients on DOAC will have a shorter period in which they are without AC protection. In case of procedures associated with low/moderate BL risk, DOAC should be discontinued a day before surgery and administered again one day following the surgical procedure. Total interruption time is two days. In case of procedures associated with high BL risk, DOAC should be discontinued two days before surgery and administered again two days following the surgery. Total interruption time is four days. In individuals on dabigartran with CrCl of 30-50 ml/min one additional day is required for low/moderate BL risk interventions and two additional days for high BL risk interventions (26). No dose adjustment is required for other DOACs (apixaban, rivaroxaban, edoxaban). Due to rapid cessation and rapid onset of action bridging is not necessary. It is recommended for ones at high risk of postoperative TE who require extended interruption of therapy (e.g. postoperative ileus after abdominal surgery) (Figure 1) (6, 13, 23).

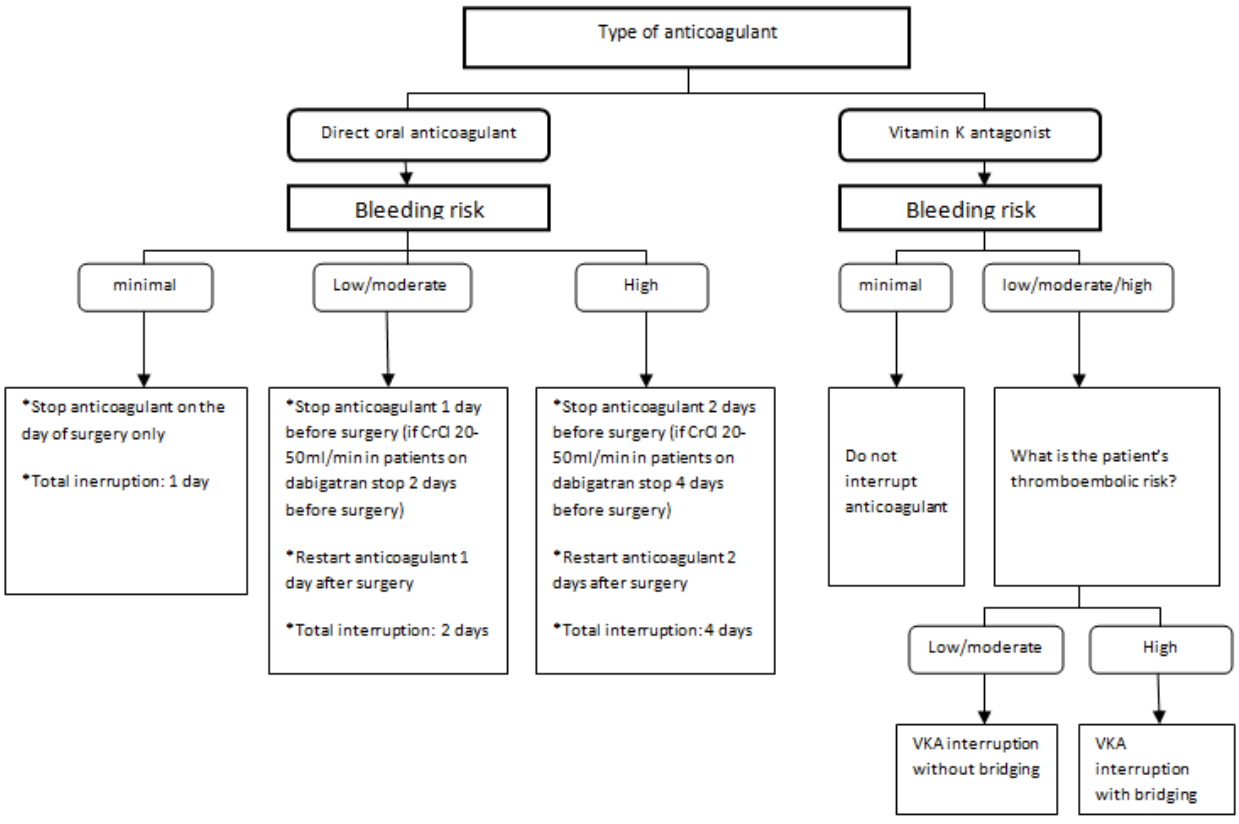

Figure 1. Algorithm for stopping anticoagulant therapy before surgery

Bridging in patients on anticoagulants

Bridging can be defined as the administration of a short-acting AT drug, most commonly a low molecular weight heparin (LMWH) or unfractionated heparin (UH) during the discontinuation period of a long-acting AT agent, most commonly warfarin. The purpose of bridging is to shorten the period during which AC coverage is absent and thus minimize the risk of perioperative TE event. However, a balance must be struck with the possibility of postoperative bleeding. Avoid bridging in patients with low TE risk like in routine prophylaxis in AF; in patients on DOACs, unless TE risk is high and postoperative period during which they cannot take DOACs is prolonged and in secondary prophylaxis of VTE which was more than 3 months ago. Bridging is advised when vitamin K antagonist is discontinued in the following cases: mitral MHV, aortic MHV with additional risk factors; stroke in the last three months or very high risk of stroke (CHADS2 score 5 or 6); VTE in the last 3 months; stent implantation in the last 3 months; previous TE during cessation of the therapy (12).

Heparin products and dosage

LMWH is most commonly used because of its equal efficacy as UH and greater reliability. Also, they generally do not require monitoring. UH is cheaper, its effects can be easily reversed and does not necessitate dose adjustment in renal insufficiency. The dose of LMWH can be prophylactic, therapeutic or moderate. Therapeutic dose is used in individuals with a source of possible embolus (AF, MHV) or VTE in the previous month. Therapeutic dose for enoxaparin is 1 mg/kg SC once a day and for dalteparin 100 IJ/kg SC twice a day. Moderate dose is used in patients with AF or VTE in the previous month when bridging is required but there is also BL risk. Moderate dose for enoxaparin is 40 mg SC twice a day and for dalteparin 5000 IJ SC twice a day. Dose adjustment is reserved for patients with renal insufficiency and obesity. In general, prophylactic doses are not used in individuals with AF, since there is no evidence that low doses are able to prevent stroke. It can be used in those with history of VTE in the last 3-12 months. The bridging therapy with LMWH starts 3 days before surgery (2 days after discontinuation of warfarin), when INR begins to fall below therapeutic values. LMWH should be stopped 24 h before surgery, based on the LMWH half-life which is 3-5 h. For twice-daily dosing, the last dose is given on the night before surgery and for once-daily dosing, one half of the last dose is given in the morning of the day before intervention. When therapeutic doses of UH are used, IV infusion is stopped 4-5 h before surgery, since the half-life of heparin is about 45 minutes. If subcutaneous UH is used, the most common dose is 250 IJ twice a day, with the last dose administered on the night prior to surgery (27-29).

Interrupt antiplatelet therapy or not?

The decision about stopping AP therapy should not be based only on the relationship between the risk of bleeding and thrombosis, but the indication for its use and the type of therapy should also be taken into consideration.

Antiplatelet monotherapy

ASA has to be stopped before surgery in patients on aspirin for primary prevention. If it is prescribed for secondary prevention, it should not be stopped (an exception involves patients at very high BL risk and in that situation, it has to be stopped for 7-10 days prior to surgery) (30). Monotherapy with some of P2Y12 inhibitors can be replaced with ASA if a patient requires surgery with moderate BL risk. If AP monotherapy is stopped, it should be restarted in the shortest possible time.

Dual antiplatelet therapy

After coronary artery stent placement, 4-15% of patients will require non-cardiac surgery in the first year after stenting. Recent findings have suggested that prolonging DAPT beyond 12 months in patients with DES is not much more efficient than ASA monotherapy in reducing the incidence of a major adverse cardiac event (31). These patients will be at high risk of bleeding if DAPT is not stopped, at high risk of TE event if DAPT is stopped and there are consequences of delaying the surgery. Because of this complexity, a multidisciplinary approach and the team work of the cardiologist, anesthesiologist and surgeon is required (15). Whether or not AP therapy is discontinued, surgery is a proinflammatory and prothrombotic condition and the risk of thrombosis in the stent segment as well as in the rest of the coronary vasculature is increased. Therefore, it is best to postpone the surgery until the expiration of the DAPT, whenever possible. In other cases leave aspirin unless contraindicated (when risk of bleeding is very high, e.g. neurosurgery). In patients at moderate TE risk (an exception is surgery with a small risk of bleeding), stop P2Y12 inhibitors three to seven days before the intervention (ticagrelor–three to five days, clopidogrel–five days and prasugrel–seven days). They should resume the therapy as early as possible, preferably 24 hours after intervention. In patients with high risk of TE event strategy will depend on BL risk. If the BL risk is low, DAPT should not be stopped. Cases with moderate and high risk of bleeding are more complicated and require individual evaluation by a multidisciplinary team. Non-cardiac surgery should be postponed until DAPT is over, unless this poses a functional risk or is life-threatening to the patient. This postponement should last six months after myocardial infarction or after stent implantation with a high risk of thrombosis. If it is not possible, it is recom-mended to postpone non-cardiac surgery for at least one month, no matter the indication or the type of stent.

If it is not possible to postpone surgery for even a month, the intervention needs to be done in a hospital with catheterization room. If both AP drugs are excluded within the first month of stent placement, a bridging strategy with some of the intravenous AP drugs may be considered. NSAIDs should not be used in the perioperative period (Figure 2) (20, 32, 33).

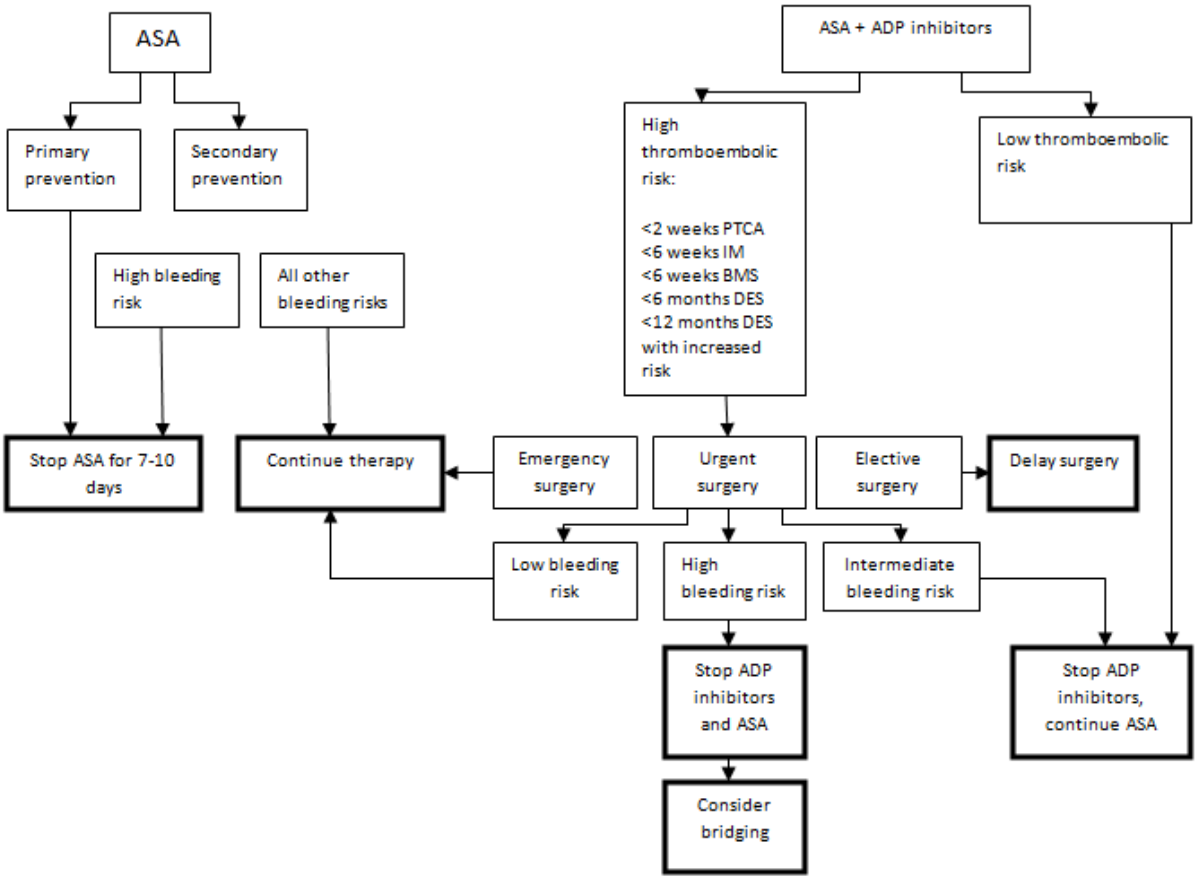

Figure 2. Algorithm for preoperative management of antiplatelet therapy

Timing of antiplatelet interruption

When ASA is prescribed for AF or primary prevention of myocardial infarction, therapy may be discontinued seven to ten days prior to surgery, while in those taking ASA for secondary prevention, discontinuation of therapy carries an increased risk of complications from the cardiovascular system. It has not been noted that perioperative ASA therapy causes increased bleeding or increased mortality, except in operations with a high BL risk. Therefore, the 2012 ACCP guidelines on perioperative management of antithrombotic therapy recommended not to discontinu ASA during the perioperative period in patients at high risk of cardiovascular events (5). Like ASA, when used for primary prevention of the cardiovascular or cerebrovascular event or for the AF, ADP inhibitors may be stopped preoperatively without major sequelae. ADP receptor inhibitors are most commonly administered before and after PCI with stent placement. In such patients, who undergo elective surgery, an adequate period of time after stent implantation is required for the surgery to be performed safely. Three to seven days before surgery is enough to reverse the effect of the ADP inhibitors (ticagrelor–three to five days, clopidogrel–five days and prasugrel–seven days) and they should be replaced with ASA whenever possible (14, 20).

Bridging in patients on antiplatelet therapy

There are few published studies and scarce clinical experience regarding bridging in patients who are taking AP drugs. Only when urgent surgery associated with a moderate or high BL risk is required and the patient has a high risk of thrombosis, the therapy can be continued. If the bridging is necessary, the recommendation is to use antithrombotics over AC drugs. Antithrombotic agents that have been studied so far are glycoprotein IIb/IIIa inhibitors (eptifibatide and tirofiban) and

P2Y12 inhibitor (cangrelor). Glycoprotein IIb/IIIa inhibitor should be started 72 hours after discontinuation of P2Y12 inhibitor. The recommended dose for tirofiban is 0.1 mcg/kg/min and 2 mcg/kg/min for eptifibatid. It should be discontinued four to six hours prior to surgery (34). P2Y12 inhibitor cangrelor should be started at least 48 hours after discontinuation of P2Y12 inhibitor and stopped 1-6 hours before surgery. The recommended dose is 0.75 μg/kg/min (34, 35).

Neuraxial anesthesia

One of the most serious complication of spinal and epidural anesthesia is spinal and epidural hematoma. Epidural anesthesia carries a higher risk, especially if a catheter is inserted. Because of increased BL risk, this type of anesthesia should not be used in patients on AT drugs. This risk is increased during catheter insertion as well as catheter removal. If neuraxial anesthesia is indicated, plan about stopping AT drugs must be made. The following recommendations are based on the 2022 guidelines for regional anesthesia in patients on antithrombotic drugs published by the European Society of Anesthesiology (36).

1) VKA: when INR is < 1.5.

2) DOACs: in low doses: last dose should be given minimum of 24 h for rivaroxaban and edoxaban, for apixaban 36 h and 48 h before puncture for dabigatran. If DOACs are administered in high doses, the last dose should be 72 h before puncture at least. In patients with impaired renal function (CrCl < 50 ml/min in high dose dabigatran therapy or CrCl < 30 ml/min in high dose direct anti Xa inhibitor therapy) preoperative laboratory test should be performed.

3) If LMWH is administered in low dose, then the last one should be at least 12 h before puncture. It is recommended that the low dose of LMWH should be halved or the last dose should be administered 24 h before the procedures in case CrCl < 30 ml/min. If LMWH is administered in high doses, the last dose should be given at least 24 h before puncturing. If LMWH is administered in high doses and CrCl < 30 ml/min the last dose should be administered 48 h before puncturing or dose should be halved.

4) For low doses of SC UH, the last dose should take place 4 h before puncture. When patient is receiving high doses of SC UH, aPTT have to be within normal range before puncture and last dose should take place 12 h before puncture. When patient is receiving high doses of IV UH last dose should take place 6 h before puncture.

5) Aspirin in doses < 200 mg is not contraindicated for performing neuraxial blockade.

6) P2Y12 inhibitors: in patients on ticagrelor the last dose should be given at least 5 days before the procedure, 5-7 days in patients on clopidogrel and 7 days in patients on prasugrel.

Conclusion

AT management plan should be made to reduce the risk of thrombosis on the one hand and bleeding on the other hand. For patients who have a VKA in their therapy that needs to be discontinued, a decision has to be made whether only withholding the AC drug would be enough or perioperative bridging with a short-acting agent is needed. Physicians' preference for routine perioperative bridging during chronic anticoagulation interruption has to be avoided. DOACs have shorter half-lives, but the laboratory tests for their activity and reversal agents is still hard to reach. In some of them, such as dabigatran, the drug effect is prolonged if renal insufficiency is present. Aspirin does not need to be stopped if the patient receives it for secondary prevention, exceptions are procedures with very high BL risk. It is best to postpone the surgery until the expiration of the DAPT, whenever possible. One of the most serious complication of spinal and epidural anesthesia is spinal and epidural hematoma. It is important to determine adequate timing of AT administration in relation to neuraxial anesthesia. The effects of AT agents should be withdrawn. Catheter manipulations and removal carry the same risk as insertion and the same criteria are used. Renal function must be considered for dabigatran and LMWH.

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Pregledni rad **UDC: 616-089.163-083+616-005.6-085:615.27.53**
doi:10.5633/amm.2022.0409

PREOPERATIVNA PRIPREMA BOLESNIKA NA HRONIČNOJ ANTITROMBOTIČKOJ TERAPIJI ZA ELEKTIVNU NESRČANU HIRURGIJU

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Preoperativna priprema bolesnika na antitrombotičkoj terapiji (antiagregaciona i antikoagulantna terapija) izazovna je, zbog toga što prekid terapije nosi rizik od tromboembolijskog događaja, a operacija je povezana sa rizikom od krvarenja. Mora se napraviti balans između tromboembolijskog rizika i rizika od krvarenja i doneti odluka o tome da li će antitrombotička terapija biti prekinuta. Svaki bolesnik zahteva individualan pristup. Ovo znači izračunavanje tromboembolijskog rizika i rizika od krvarenja za svakog bolesnika. Rizik od krvarenja je zasnovan na individualnim karakteristikama bolesnika i riziku koji nosi sama hirurška intervencija. Tromboembolijski rizik je kompleksniji za izračunavanje. Ukoliko je doneta odluka da se prekine antitrombotička terapija, sledeće pitanje je koliko pre operacije treba biti prekinuta i da li neophodno premošćavanje.

Acta Medica Medianae 2022;61(4):63-72.

Ključne reči: *antitrombotička terapija, tromboembolijski rizik, rizik od krvarenja*

Review article **UDC: 802.0-3:61**
doi:10.5633/amm.2022.0410

THE IMPLEMENTATION OF STRATEGIES FOR ENHANCING STUDENTS' COMMUNICATIVE COMPETENCE IN MEDICAL ENGLISH COURSE

Nataša Šelmić

In the contemporary world, English is considered to be the dominant language of communication in the field of medicine. The aim of this paper is to present specific features of the Medical English course indispensable for the successful education and professional development of medical students. It is believed that an integrative and interdisciplinary approach in which the student is at the centre of the teaching/learning process is the fundamental aspect of the Medical English course Therefore, students should be encouraged to adapt and upgrade the language skills applicable in future professional setting. The English language learning strategies at the tertiary level include acquiring medical terminology, upgrading oral and written communication, and following medical literature. Moreover, Medical English practitioners should combine different approaches based on their experience leading to the most appropriate teaching outcomes.

Acta Medica Medianae 2022;61(4):73-76.

Key words: *Medical English, medical terminology, professional development, teaching/learning process, language skills*

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Introduction

In the contemporary world, English is considered to be the dominant language of communication in the field of medicine. It is used as the primary means of communication between medical experts, which leads to the uniformity of science and scientific language. It is characterized by specific linguistic features and requires a specialized language teaching methodology which can be applied by using a specifically designed programme (1).

It is believed that an integrative and interdisciplinary approach in which the student is at the centre of the learning process is the fundamental aspect of teaching English for medical purposes (EMP). This approach is based on the authenticity and cooperation between the EMP practitioner and the students, as well as between the students themselves (2). In addition, students should be prepared for long-term learning, i.e. learning that will continue after the completion of the Medical English course.

Fundamental features of English for medical purposes course

The English for medical purposes course needs to satisfy the requirements of modern methodological principles at the tertiary level in being a student-centred approach directed towards the interests of the students and their future professional careers. The goals of EMP teaching generally include broadening content-based knowledge and developing the ability to communicate using appropriate linguistic tools.

Therefore, it is very important to begin the process of designing an adequate programme by performing the needs analysis that will determine the current level of knowledge, motivation, and previous learning methods. Accordingly, this information will help the EMP practitioner adjust the curriculum using different methodological approaches (3).

In this context, the role of the EMP practitioner is to prepare students for their future studies and enhance their motivation. This implies that medical students should adopt specific medical vocabulary, improve oral and written communication in English, and apply the language in various work-related situations (4).

Communicative tasks in EMP teaching

Communication tasks that await students in the professional future include:

• The interaction with professionals including cooperation with medical staff, writing and presenting case reports, referring to a specialist, and professional consultations;

• The interaction with patients consisting of history taking, performing a physical exam, explaining diagnostic procedures and medical conditions, and establishing treatment plans;

• The academic interaction encompassing reading and writing journal articles, participating in conference presentations, and discussions;

The instruction in any of the above categories may form the solid basis of an EMP curriculum that is in accordance with the students' needs (5).

Task-based language teaching

Task-based language teaching (TBLT) focuses on performing different tasks using the target language. The evaluation is based more on the appropriate achievement of real-life tasks than on the correctness of the given language structures. In this way, students develop target language fluency and strengthen self-confidence (6). The main elements of a task include pragmatic meaning; the information gap, the reasoning gap, the opinion gap, and linguistic resources needed to complete the task (7).

It is commonly believed that task-based learning consists of three stages:

• a *pre-task stage* in which the topic is defined and the students participate in activities that enable them to acquire the vocabulary relevant for the accomplishment of the main task;

• a *task cycle* in which the students perform the task in pairs or small groups and then prepare a report in which they explain how they completed the task, and

• *the language focus stage* in which specific language features are highlighted.

The aim of such a model is to make students incorporate all four language skills and become more fluent and accurate. The language is used in the medical context and the emphasis is on communication (8).

The use of simulation in EMP teaching

Following the principles of the student-centred approach, the aim of simulations is to make students active participants in the teaching/learning process. In other words, students experience the activity directly rather than hearing about it. The simulation is open-ended, implying a variety of possible answers to the problems students are required to solve. Therefore, simulations depend on the decisions made and the actions taken.

Simulations reflect a real-life situation in which students undertake roles as they analyze data, make decisions and solve the problems essential to the given situation. As the simulation proceeds, students respond to the changes within the situation by analyzing the ramifications of their decisions and subsequent actions and predicting future problems (9).

Simulations motivate students by keeping them engaged in the problem-solving and decision-making. In addition, they advocate critical thinking and help students upgrade knowledge by actively participating in student-student or practitioner-student conversations needed to conduct a simulation and transfer knowledge to new problems and situations (10).

Problem-based learning

Problem-based learning (PBL) focuses on the integration of language and content study to facilitate autonomous learning. The underlying principle is that students learn better if the presented content is familiar to them. The PBL emphasizes the acquisition of content knowledge aiming at a high level of communication among learners of the same group. The PBL was initially developed for medical education and has then been extended to other disciplines. The process allows students to develop skills used for their future practice.

The PBL tutorial process involves working in small groups of students. Each student takes on a role within the group that may often revolve. It is directed towards the student's reflection and reasoning to create their learning environment (11). Students should be encouraged to take on the problem, and develop and improve strategies for individual learning and team work (12). In this way, they increase responsibility for their learning which will help them to further progress in their primary field of expertise.

Project-based learning

Project-based learning emphasizes authentic learning tasks established in the personal interests of learners. It is a systematic teaching method that engages students in acquiring knowledge and skills through an extensive investigation constructed around intricate and meticulously selected issues (13).

In the context of EMP, project-based learning aims at bringing the workplace environment into the lectures (14). The project work enables the students to put their medical knowledge into the context of English, which is a source of great motivation. The best projects call upon the prior knowledge and expertise of each student. They are aware of the purpose and relevance since they acquire the knowledge necessary for their future area of expertise. In the process of preparing the projects, students may consult experts in the core courses and come up with great ideas. An example of the project - based learning would be asking students to search for information and make a comparison between healthcare systems in different countries of the European Union and their own country.

Conclusion

Due to the rapid development of medical science and language teaching methodology, Medical English teaching represents a challenging job requiring myriad skills from language instructors. Not

only should they be facilitators of the teaching process creating conditions for learning, but they should also assist students in acquiring the knowledge necessary to promote their professional development. In line with this, designing a Medical English course is a highly motivating process calling for the productive exchange of ideas between teachers and students, close collaboration with teachers of the core courses, and continuous evaluation to create a curriculum specially designed for the medical profession. Moreover, Medical English practitioners should combine different approaches based on their experience leading to the most appropriate teaching outcomes.

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Pregledni rad **UDC: 802.0-3:61**
doi:10.5633/amm.2022.0410

PRIMENA STRATEGIJA ZA UNAPREĐENJE KOMUNIKATIVNE KOMPETENCIJE STUDENATA U NASTAVI ENGLESKOG JEZIKA ZA POTREBE MEDICINE

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Smatra se da je engleski jezik danas dominantni jezik komunikacije u oblasti medicine. Cilj ovog rada je da predstavi specifičnosti nastave engleskog jezika u oblasti medicine, neophodne za uspešno obrazovanje i profesionalni razvoj studenata medicine. Integralni i interdisciplinarni pristup, u kojem je student u centru procesa učenja, predstavlja osnovni aspekt nasatve engleskog jezika za potrebe medicine. Zbog toga studente treba podsticati da usvajaju i usavršavaju jezičke veštine primenljive u budućem profesionalnom okruženju. U strategije učenja engleskog jezika na akademskom nivou spadaju usvajanje medicinske terminologije, savladavanje usmene i pismene komunikacije, kao i praćenje medicinske literature. Štaviše, profesori engleskog jezika za potrebe studenata medicine treba da kombinuju različite pristupe zasnovane na svom iskustvu, koji bi doveli do najboljih mogućih rezultata.

Acta Medica Medianae 2022;61(4):73-76.

Ključne reči: *medicinski engleski jezik, medicinska terminologija, profesionalni razvoj, jezičke veštine*

Review article

UDC: 615.322:582(497.11)
doi:10.5633/amm.2022.0411

HERBAL DRUGS AND TRADITIONAL HERBAL DRUGS IN THE REPUBLIC OF SERBIA

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According to the Law on Medicines and Medical Devices of the Republic of Serbia, which is harmonized with the European directives on medicines, a substance that is an active component of a medicine can be of plant origin. Herbal medicine - HM and traditional herbal medicine - THM are used for the prevention and treatment of certain diseases and conditions, their initial, mild, but also chronic and recurrent forms.

The guidelines on the pharmaceutical quality of HM and THM insist on a rigorous and detailed definition of plant raw materials, production process, and finished pharmaceutical products. Registration of these types of drugs is done by the Agency for Drugs and Medical Devices of Serbia (ALIMS). During the HM registration process, the Herbal Anatomical Therapeutic Chemical (HATC) system (an integral part of the WHO Drug Global Classification of products and substances) is applied. For now, the number of registered HMs and THMs in Serbia is modest in comparison to their number in most EU member states. ALIMS controls the content of the patient information leaflet for the medicine: all data on indications, dosage, contraindications, precautions, adverse effects, and interactions. Therefore, the application of registered HMs and THMs constitutes a modern phytotherapeutic approach and the safest way to use herbal medicinal substances and preparations.

In addition to medicinal, there are other categories of herbal products on the market (herbal supplements, herbal teas…) that are not intended for therapy and treatment. Dietary products are regulated by other laws and regulations, and after entry in the Register of Dietary Products of the Ministry of Health, they can be put in the Serbian market.

Acta Medica Medianae 2022;61(4):77-86.

Key words: *herbal medicines, traditional herbal medicines, Serbian market*

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Introduction

The use of plants for therapeutic purposes has a very long tradition. Today, both in developed and developing countries, there is a great interest in herbal medicinal products due to the numerous biological and pharmacological activities that can be manifested (1-3).

Phytotherapy is a treatment system based on the application of herbal medicinal products. It belongs not only to pharmacotherapy and conventional medicine, but also to traditional (folk) medicine, and complementary and alternative medicine (2, 4).

Rational phytotherapy encompasses the treatment, alleviation, and prevention of diseases and health problems with herbal medicines (herbal medicinal products of scientifically and clinically proven therapeutic efficacy – *evidence-based herbal medicines*). It is evidence-based phytotherapy (1, 5-7). However, with the increasing use of different categories of plant products in recent decades, the issue of safety of their application has become increasingly important. Although they usually have a favourable risk-benefit ratio, not all of them can be considered completely safe. Like conventional drugs, they can have adverse effects and/or interact with other substances. The pharmacovigilance of herbal medicinal products is a great challenge not only because of their unique characteristics, but also because of the way in which they are legally regulated, used and accepted (1-3).

The aim

To clarify the place and position of herbal medicinal products, this paper will present data on herbal medicines and traditional herbal medicines

from the markets of European countries and the Republic of Serbia, with special reference to data on their HATC (Herbal Anatomical Therapeutic Chemical Classification) and indication area. Further will be discussed pharmaceutical forms and pharmacovigilance of herbal medicines, but also their differences from other categories of herbal products on the market.

Results and discussion

Legislation currently in force

According to the Law on Medicines and Medical Devices of the Republic of Serbia, which is harmonized with the European directives on medicines (65/65 EEC and 2001/83/EC), a substance that is an active component of a (human) medicine can also be of plant origin (5, 8-10). Further, the Law of Republic of Serbia recognizes two types of drugs with an active component of herbal origin: herbal medicines and traditional herbal medicines (8).

It is good to be known, that terms "herbal substance" and "herbal preparation", which are used in the legal acts of the Republic of Serbia and European regulatory documents (documents published by EMA – the European Medicines Agency), correspond to the terms "herbal drug" and "herbal drug preparation", which are used in pharmacopoeias (for example European Pharmacopoeia – Ph. Eur.) and documents of the World Health Organization (WHO)) (5, 8-13).

A herbal medicine (HM) is "any medicine whose active ingredients are exclusively one or more (substances of plant origin) herbal substances or one or more herbal preparations or one or more herbal substances in combination with one or more herbal preparations" (8).

Traditional medicines are the result of tradition or other traditional therapeutic approaches and can be based on scientific principles. A traditional herbal medicine (THM) is "a medicine that has indications characteristic exclusively for THMs, which by their composition and purpose are intended for use without medical supervision, exclusively in accordance with the prescribed strength and dosage for oral or external use or inhalation, and for which there is sufficient data on the traditional use of the drug, or it has been shown that it is not harmful under the prescribed conditions of use as well as that its pharmacological effects or efficacy can be expected based on its long-term use and experience". If it contains vitamins or minerals of well-documented therapeutic safety, it can be considered a THM if the effect of these vitamins or minerals is only ancillary to the action of the active herbal ingredients in terms of the established indication or indications (7, 8, 10, 14).

The category of herbal medicines/traditional herbal medicines does not encompass dosed pharmaceutical forms in which the active ingredient is an isolated compound or mixture of pure substances isolated from herbal raw materials (e.g., digitoxin, lanatoside C, atropine, nicotine, morphine, codeine, vinca alkaloids, taxanes, podophyllotoxins) – these are conventional drugs (1, 6, 8).

Pharmacopoeias prescribe quality requirements and, within them, the health safety of herbal drugs and their preparations, i.e., substances of plant origin (15). Before being marketed, HMs and THMs must meet all the criteria concerning safety, quality, and efficacy, just like all other medicines. The HM and THM registers are maintained by the Agency for Medicines and Medical Devices of Serbia (Ser. Agencija za lekove i medicinska sredstva Srbije, ALIMS).

In order to market medicines with an active component of plant origin, both Serbia and the European Union offer three procedures for product registration – two for HMs and one for THMs (1, 14, 16) (Figure 1). The Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMA) publishes EU monographs on herbal medicinal substances, which comprise preclinical and clinical data on HMPs with well-established use (WEU) as well as data on traditional use (TU) (7). Herbal monograph with WEU/TU is a solid base for registration of an HM/THM.

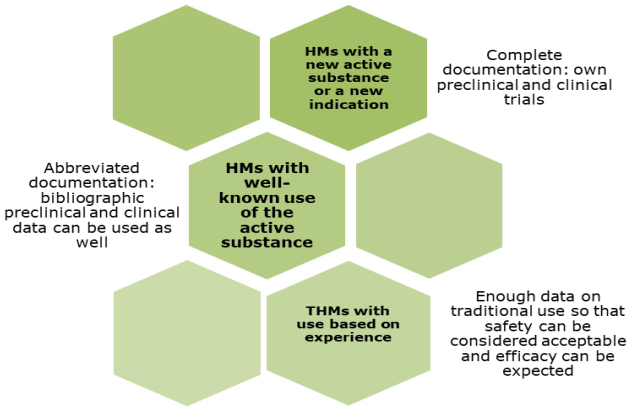

Figure 1. Registration procedures and requirements to be met by HMs and THMs in addition to certified pharmaceutical quality

Herbal Anatomical Therapeutic Chemical Classification (HATC)

The HM and THM quality guidelines insist on a rigorous and detailed definition of the starting plant materials – specific botanical identification includes the binomial nomenclature (species, genus, variety, and author), chemotype (when significant), name of the part of the plant used, knowledge of the location where the plant grows, and conditions for obtaining plant raw materials. Insisting on binomial nomenclature and the HATC (Herbal Anatomical Therapeutic Chemical) classification for HM is extremely important since many plant species have several folk names and one folk name often refers to several different plant species (which are not related and whose effects differ) (1, 17).

The HATC (Herbal ATC) system is an integral part of WHODrug Global for the classification of products and substances. It provides a classification of HMs according to the internationally approved classification of the Latin binomial name and common therapeutic use. It enables the collection, grouping, and aggregation of HM data at different levels of specificity. As with the ATC system, HMs in HATC are divided into groups according to therapeutic use. The first level consists of 14 anatomical groups marked with the letters A–V (the same in ATC and HATC). The following levels are similar in the two classifications, but in some cases, additional categories are introduced in the HATC for specific groups of plants. For example, a complete HATC classification of the Aloe ferox Mill. preparation, used as a laxative, is A06AB5001: A – alimentary tract and metabolism (level 1, main anatomical group); A06 – drugs for constipation (second level group, main therapeutic group); A06A – drugs for constipation (third-level group, therapeutic/pharmacological subgroup); A06AB – contact laxatives (fourth-level group, therapeutic/pharmacological/ chemical subgroup); A06AB5001 – *Aloe ferox* Mill., dry leaf juice (fifth-level group, individual raw herbal substance) (17).

Registers of herbal medicines and traditional herbal medicines in the Republic of Serbia

The total numbers of registred HMs and THMs in the RS (Table 1 and Table 2) are modest in comparison to their numbers in the EU. German market counts total of 1008 herbal medicinal products with completed marketing authorisation or registration procedure, from which 841 are single component and 167 fixed herbal combinations. Traditional herbal medicinal products with completed registration procedure in Germany counted in total 293 THMs in March 2022 (173 single component and 120 fixed combinations) (18). Germany is a leader in phytotherapy application among the EU members' countries. According to the latest survey (status December 31 2016) of Inspections, Human Medicines, Pharmacovigilance and Committees Division of EMA Germany definitely had the greatest total number (447) of "well-established use" medicinal product applications in EU Member States, while the order of numbers for "traditional use" medicinal product applications was 513, 450 and 315 in Germany, UK and Poland, respectively (19). In Serbia 38 HMs and 24 THMs were registered at the beginning of 2017 while in 2016, there was 1 THM less (20, 21). Since then, the total number of HM and THM has fallen from 62 to 50 (22).

Table 1. Herbal medicines in the Republic of Serbia (in June 2022) (22)

HATC	Herbal medicine	Active substance	HATC	Pharmaceutical form
HA ALIMENTARY TRACT AND METABOLISM				
HA03A medicine for functional bowel disorders	IBEROGAST	Bitter candytuft (*Iberis amara* L.), liquid extract of fresh whole plant, Angelica (*Angelica arhangelica* L.), liquid root extract, Lemon balm (*Mellissa officinalis* L.), liquid leaf extract, Caraway (*Carum carvi* L.), liquid fruit extract, Celandine (*Chelidonium majus* L.), liquid herb extract, Liquorice (*Glycyrrhiza glabra* L.), liquid root extract, Chamomile (*Matricaria recutita* L.), liquid flower extract, Peppermint (*Mentha piperita* L.), liquid leaf extract, Milk thistle (*Silybum marianum* L.), liquid fruit extract	HA03A	Oral drops 20 ml; 50 ml
BILE AND LIVER THERAPY				
A05BA PREPARATIONS IN THE TREATMENT OF LIVER DISEASE	ESSENTIALE® FORTE N	essential phospholipids 300 mg	A05BA	Hard capsules 3x10 pcs
	ESSENTIALE® MAX	600 mg		Hard capsules 5x6 pcs
LAXATIVES				
HA06AB CONTACT LAXATIVES	BEKUNIS	Senna alexandrina pods (*Sennae fructus angustifoliae*), dry aqueous extract	HA06AB06	Gastro-resistant tablets, 1x10 pcs and 1x45 pcs Instant herbal teas, 1x17.6 g
	BEKUNIS	Senna (*Cassia senna/Cassia angustifolia*), leaf	HA06AB06	Herbal teas,1x80 g

A06AC LAXATIVES THAT INCREASE THE VOLUME OF INTESTINAL CONTENTS	MUCOFALK® POMORANDŽA	Ispaghula Husk (*Plantago ovata*)	A06AC01	Granules for oral suspensions 20x5 g
	TRANSILANE			Powder for oral suspensions 20x7 g
RESPIRATORY SYSTEM				
COUGH AND COLD MEDICINES R05	BRONCHIPRET	Thyme (*Thymus vulgaris* L. and/or *Thymus zygis* L.), liquid herb extract, Ivy (*Hedera helix* L.), liquid leaf extract	HR05WA	Oral liquid 50 ml and 100 ml
	HERBION®LOZENGE OD BRŠLJANA	Ivy (Hedera helix L.), dry leaf extract	R05CA12	Compressed lozenges 2x8 pcs, 3x8 pcs and 4x8 pcs
R05CA EXPECTORANTS	BRONHOKLIR SYRUPS® BRŠLJAN	Ivy (*Hedera helix* L.), dry leaf extract	R05CA12	Syrup 125 mL**
	HEDELIX			Oral drops, Syrup 100 mL
	MUCOPLANT SIRUP ZA KAŠALJ SA EKSTRAKTOM BRŠLJANA			Oral liquid 100mL and 250 ml
	PROSPAN			Effervescent tablets 5x2 pcs, Pastilles 2x10 pcs, Syrup 100 mL and 200 mL
	PROSPAN® KAPI			Oral drops 20mL
	PROSPAN LIQUID			Oral liquid 15x5 mL, 21x5 mL, 100 mL and 200 mL
	TUSPAN®			Syrup 120 mL**
HR05WA DIAPHORETIC HERBS AND OTHER COUGH AND COLD MEDICINES	HERBION® SIRUP OD BRŠLJANA	Ivy (*Hedera helix* L.), dry leaf extract	HR05WA	Syrup 150 mL
HR07A OTHER COUGH AND COLD MEDICINES	GELOMYRTOL® FORTE	Distillate obtained from the mixture of rectified essential oils of eucalyptus, sweet orange, myrtle, and lemon, standardized at 1,8-cineole, d-limonene i (+)-alpha-pinene 300mg	HR07A	Gastro-resistant tablets 2x10 pcs
R05X OTHER COMBINED COUGH AND COLD MEDICINES	SINUPRET® FORTE	Dry extract derived from, flowers, herb, flower, and herb: Verbena (*Verbena officinalis* L.), powdered herb, gentian (*Gentiana lutea* L.), powdered root, sorrel (*Rumex acetosa* L.), powdered herb, elder (*Sambucus nigra* L.), powdered flower, primula (*Primula veris* (L.) and/or *Primula elatior* (L.) Hill), powdered flower and bud	R05X	Coated tablets 1x20 pcs
	SINUPRET®AKUT			Coated tablets 2x10 pcs
	SINUPRET®SIRUP			Syrup 100mL
PSYCHOLEPTICS				
N05CM OTHER HYPNOTICS AND SEDATIVES	PERSEN® NIGHT	Valerian (*Valeriana officinalis* L.), dry root extract	N05CM09	Coated tablets 2x15 pcs
NERVOUS SYSTEM				
N06DX OTHER MEDICINES FOR THE TREATMENT OF DEMENTIA	BILOBIL	Ginkgo (*Ginkgo biloba*), dry leaf extract	N06DX02	Capsules 40 mg 2x10 pcs and 6x10 pcs
	BILOBIL FORTE			Capsules 80 mg 2x10 pcs and 6x10 pcs
	BILOBIL INTENSE			Capsules 120 mg 2x10 pcs
	TANAKAN		HN06DX	Coated tablets 2x15 pcs** 6x15 pcs**
ANTIBIOTICS AND CHEMOTHERAPEUTICS FOR DERMATOLOGICAL USE				
D06BB ANTIVIRAL DRUG	VEREGEN	Green tea (*Camellia sinensis* (L.) O. Kuntze), dry leaf extract	D06BB12	Ointment 1x15 g

* The same HM in the different package was considered as one medicine;
** one HM produced by 2 different manufacturers were considered as one medicine.

Table 2. Traditional herbal medicines in the Republic of Serbia (in June 2022) (22)

THM	Active substance	Pharmaceutical form	Traditional use
ARNIKAMED DOLO	Arnica (*Arnica montana* L.) flower tincture	Gel 50 g and 100 g	relief of bruises, sprains and localised muscular pain
BRONCHICUM® SIRUP S	Thyme (*Thymus vulgaris* L. and/or *Thymus zygis* L.), liquid herb extract	Syrup 100 mL	expectorant in cough associated with cold
BRONCHOSTOP® PASTILE	Thyme (*Thymus vulgaris* L./*Thymus zygis* L.), dry herb extract	Pastilles 2x10 pcs	expectorant in cough associated with cold

BRONCHOSTOP® SINE SIRUP	Thyme (*Thymus vulgaris* L./*Thymus zygis* L.), dry herb extract, marshmallow (*Althaea officinalis* L.), liquid root extract	Syrup 120 mL	demulcent for the symptomatic treatment of oral or pharyngeal irritation and associated dry cough
CANEPHRON®	Common centaury (Centaurium erythraea Rafn s.l.), powdered herb, Lovage (Levisticum officinale Koch.), powdered root, Rosemary (Rosmarinus officinalis L.), powdered leaf	Coated tablets 2x15 pcs	prevention of recurrent formation of kidney stones and relief of symptoms of recurrent mild lower urinary tract infections in women
CARSIL	Milkthistle (*Silybum marianum* L. Gaertner), dry fruit extract	Capsules 5x6 pcs, Coated tablets 8x10 pcs	alleviation of liver dysfunction, cirrhosis of the liver, and toxic liver damage
CYNARIX	Artichoke (*Cynara scolymus* L.), dry leaf extract	Coated tablets 2x12 pcs	symptomatic relief of digestive disorders
FAVORA® SILYMARIN	Milkthistle (*Silybum marianum* L. Gaertner), dry fruit extract	Capsules 3x10 pcs	adjuvant therapy of chronic inflammatory liver disease, cirrhosis of the liver, and toxic liver damage
HERBION® SIRUP OD BOKVICE	Ribwort Plantain (*Plantago lanceolata*), liquid leaf extract, Mallow (*Malva sylvestris*), liquid flower extract, ascorbic acid	Syrup 150 mL	symptomatic treatment of dry, irritating cough
HERBION® SIRUP OD ISLANDSKOG LIŠAJA	Iceland moss (*Cetraria islandica*), soft thallus extract	Syrup 150 mL	for oral or pharyngeal irritation and associated dry cough
HERBION® SIRUP OD JAGORČEVINE	Primula (*Primula veris* L./*Primula elatior* Hill.), liquid root extract, Thyme (*Thymus vulgaris* L./*Thymus zygis* L.), liquid herb extract	Syrup 150 mL	expectorant in cough associated with cold
MUCOPLANT	Ribwort Plantain (*Plantago lanceolata*), liquid leaf extract	Syrup 100 mL and 250 mL	symptomatic treatment of oral or pharyngeal irritation and associated dry cough
PERSEN	Valerian (*Valeriana officinalis* L.), dry root extract, Lemon balm (*Melissa officinalis* L.), dry leaf extract, Peppermint (*Mentha piperita* L.), dry leaf extract	Coated tablets 4x10 pcs*	relief of mild symptoms of mental stress and help with insomnia
PERSEN® FORTE		Hard capsules 2x10 pcs	
PERSEN® FORTE N	Valerian (*Valeriana officinalis* L.), dry root extract, Passionflower (*Pasiflora incarnata* L.), dry herb extract	Capsules 2x10 pcs	relief of mild transient nervous tension and anxiety due to mental stress, and temporary difficulty sleeping
PROSTAMOL® UNO #	Saw Palmetto (*Serenoa repens* (Bartram) Small), soft fruit extract	Soft capsules 2x15 pcs and 4x15 pcs	relief of symptoms related to benign prostatic hyperplasia
ROWIREN	Rosemary Oil (*Rosmarinus officinalis* L.), essential oil	Cream 50 g and 90 g	relief of minor muscular and articular pain and in minor peripheral circulatory disorders (with symptoms such as cold feet)
TRIBESTAN	Tribulus (*Tribulus terrestris* L.), dry herb extract	Film tablets 6x10	in treatment of decreased libido and impotence in men
TUSSAVIT	Thyme (*Thymus vulgaris* L. and/or *Thymus zygis* L.), liquid herb extract, Ribwort Plantain (*Plantago lanceolata* L.), liquid leaf extract	Syrup 250 g	expectorant in cough associated with cold

* The same THM in the different package was considered as one medicine;
** one THM produced by 2 different manufacturers were considered as one medicine;
# Although the Agency for Drugs and Medical Devices of Serbia shows it as an HM on their site, PROSTAMOL® UNO is THM. Saw Palmetto soft fruit ethanolic (ethanol 96%, V/V) extract is an herbal medicinal substance with traditional use and in the patient information leaflet, PROSTAMOL® UNO is declared as THM.

Yet, *Hederae helicis folium,* followed by *Ginkgo folium,* was the most present herbal substance in the HMs in both the RS and the EU (22, 19). The largest number of HMs and THMs registered in the Republic of Serbia are intended for use in various respiratory disorders followed by alimentary tract complaints (Figure 2).

HMs and THMs registred in RS are widely used in different mild diseases and health problems (Table 1 and Table 2). However, they are mostly not recommended for use during pregnancy and lactation, and in the population of infants and young children due to insufficient data (7).

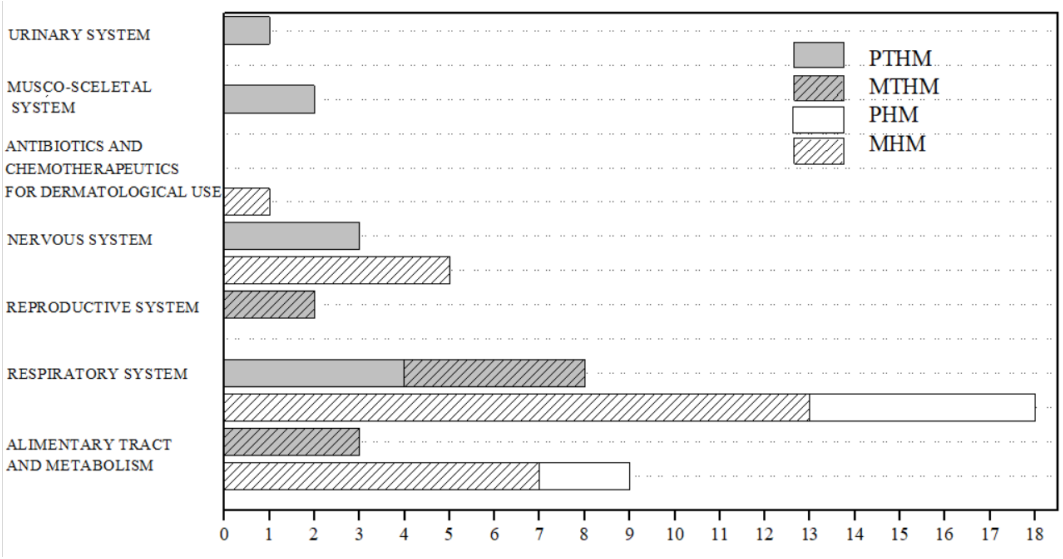

Figure 2. Number of polycomponent/monocomponent traditional herbal medicines (PTHM/MTHM) and polycomponent/monocomponent herbal medicines (PHM/MHM) by indication for use in the Republic of Serbia (in March 2022)

Pharmaceutical forms of herbal medicines

Herbal medicines and traditional herbal medicines are produced in a number of pharmaceutical forms. The first associations for herbal medicines are herbal teas. Solid dosage forms (e.g. herbal tea bags, powders, dry extract powders, granules, pills, capsules, tablets, lozenges) are most often found in the herbal medicine (23). Nevertheless, among HMs and THMs, there is only one herbal tea on the Serbian market currently (Table 1). Oral drops and liquids or syrups are made from simple tinctures as herbal drug preparations. Hard capsules may contain a powdered herbal drug or a dry most often enriched extracts, while tablets are generally produced from dry extracts. Further, liquid extracts can be included in liquid pharmaceutical forms for oral and skin application and semi-solid preparations (creams, gels) for local, rectal, vaginal, or oral mucosal application (24). The pharmaceutical forms of HMs and THMs also encompass herbal teas, instant herbal teas, ointments, film, coated, gastro-resistant and effervescent tablets, pastilles, compressed lozenges, granules, and powder for oral suspensions (Table 1, Table 2).

Medicinal herbs and consequently their products are very sensitive to external influences (temperature changes, moisture, and direct light). Since reactions of oxidation, degradation, hydrolysis, and evaporation can affect the quality of the final product, its stability, shelf life, and storage conditions must be defined (25). The quality control of these groups of medicines, in addition to the specifics of the control conditioned by the active component, also includes tests related to the pharmaceutical form in which they are produced as finished medicines or manufactured as galenic medicines (26). Also, to maintain quality of the final product, different quality parameters must be monitored for different pharmaceutical forms of HMs and THMs. Identification, appearance, and microbiological purity/safety, and/or complete health safety control are common parameters of quality control for all herbal medicinal products. Verification of components and their declared mass ratio, and verification of package weight must be done for mono-component teas and tea mixtures (25, 27). Additional parameters for liquid herbal preparations are loss on drying, the content of ethanol, relative density, and refractive index. pH value is important for semi-solid forms while solid-dosage forms also must be defined by the declared mass of single-dose preparations and disintegration (25).

Pharmacovigilance of herbal medicines

Herbal medicines are safe when properly applied and used in the recommended therapeutic doses, and the adverse effects consist primarily of mild and rare gastrointestinal problems or dermatological reactions. However, unlike most conventional medicines, which most frequently consist of a single chemically defined active substance, HMs and THMs are chemically rich, complex mixtures with hundreds of ingredients. Some HMs and THMs contain more herbal raw materials (herbal substances and preparations), which makes their composition more complex and complicates pharmacokinetic, pharmacodynamic, and toxicological studies. For many HMs and THMs, neither the exact chemical composition nor their complete safety profile is known. The chemical profile of the plant raw material varies depending on the plant organ used (not the same for the whole plant), inter - and intraspecific variations of the active compounds present, environmental factors (climate and plant growth conditions), and the harvesting/gathering time and processing procedure (storage, drying, processing, etc.).

The safety and efficacy of HMs and THMs should also be considered in the light of variations from different manufacturers and different preparations of the same herbal substance used (1, 3). The control of raw materials, intermediate products, and finished HMs and THMs is a prerequisite for quality and safe medicines on the market.

The end of the 20th century saw the establishment of the department for the adverse effects of herbal medicines at the WHO Collaborating Center in Uppsala (Global Pharmacovigilance Center). For HMs and THMs, as well as for other over-the-counter medications, doctors rarely report adverse effects, so there is not only an important role for pharmacists and the organization of a spontaneous reporting system through the pharmacy network, but also for spontaneous reporting of adverse effects by patients. Despite initiatives to encourage reporting of suspected adverse reactions associated with HMs and THMs, the number of these reports remains low compared to the number of reports for conventional medicines – only 0.5-1% of the total number of reports relate to herbal medicines (3, 28).

The history of safe traditional use provides some degree of certainty in the absence of acute toxicity, but mostly does not provide relevant information on many other safety aspects such as the effects of prolonged use, delayed and "hidden" adverse effects, impact on relatively new diseases (e.g., HIV infection), and concomitant use with conventional drugs (3).

Herbal medicinal products are widely used for maintaining health, preventing disease, and treating and self-treating chronic and recurrent conditions and diseases. They are dispensed in a pharmacy without a doctor's prescription or procured in other ways and are mostly used for self-treatment (4, 6). Such status is justified by the large therapeutic range, low toxicity, safety in overdose, minimal interactions, as well as indications that are well known to the patient/user. However, insufficient, or incorrect information on the product can lead to the occurrence of adverse effects or a delay in starting adequate therapy. When it comes to HMs and THMs, the possibility of error is very small since ALIMS controls the contents of the patient information leaflet for the medicine (which, among other things, lists all the information on contraindications, precautions, known and confirmed adverse effects and interactions) (29).

The other herbal products

Herbal products can also be found as cosmetic products for the protection and care of skin and mucous membranes, or as part of dietary products, as well as herbal teas. Although these product categories are not intended for therapy, they can be very useful if they are applied correctly. Special laws and bylaws regulate each product category. Usually, only the part related to the safety of their application is of concern to the ministry that deals with health affairs.

In the following of the study, more information will be given for certain products from the category of dietary products and one part of them: food supplements and herbal (food) supplements (30, 31).

Dietary products belong to the area of food and they are used to preserve and improve health as well as to reduce the risk of disease in later life; these products are not intended for therapy and treatment. In addition to nutritional statements, dietary products may also contain certain health statements. A health statement is any statement that states, indicates or suggests that there is a link between a category of food, a particular food or one of its ingredients and health. Also, a disease risk reduction statement is any health statement that states, indicates or suggests that consuming a certain category of food, a certain food or one of its ingredients significantly reduces the risk factor for developing the disease in humans. Only health statements approved by the Ministry of Health can be found in the dietary product declaration (32).

Until recently, there was a single rulebook that applied to all types of dietary products, but in 2022. a special rulebook regulating the area of food supplements was prepared (31).

Food supplements are foods that supplement the usual diet, and which are concentrated sources of nutrients or other ingredients with nutritional or physiological effect, individually or in combination, and are marketed in dosage forms such as capsules, lozenges, tablets and the like, powder bags, ampoules of liquid, dropper bottles, and other similar forms of liquid and powder intended for taking in small, metered quantities. "Food supplements as an active ingredient may contain herbs, herbal raw materials, herbal preparations, or ingredients isolated from herbal raw materials, individually or as a mixture" (Article 11); "When the food supplement contains plants, plant raw materials, preparations of plant raw materials or ingredients isolated from plant material, their amount in the daily dose of the product should not be less than 15% and more than 65% in relation to the known lowest therapeutic daily dose of that plant raw material or preparations, as defined in the monographs of the European Medicines Agency (EMA), the European Scientific Association for Phytotherapy (ESCOP), the World Health Organization (WHO), the German Commission E and the Physicians' Desk Reference for Herbal Medicines" (Article 12); "Food supplements must not contain plants, parts of plants or plant preparations that contain ingredients of strong pharmacological activity, regardless of their share in the product, as well as plants, parts of plants or plant preparations for which relevant data have harmful effects on human health" (Article 13) (31).

The placing on the market of such dietary products is based on other laws and regulations relating to food. In this case, there is a division of responsibilities between the Ministry of Agriculture and the Ministry of Health. The Ministry of Agriculture is responsible for the production itself, the Ministry of Health is responsible for the safety of application and health safety. Placing plant supplements on the market can be realized after entry in the Register of dietary products, for which the Ministry of Health is responsible (31).

Herbal teas can be monocomponent and multicomponent and are classified into different product categories. Thus, herbal teas can be uncategorized products, herbal food supplements, THMs, and HMs. The list of galenic medicines used in human medicine currently includes about twenty galenic medicines with herbal raw materials, and the majority of them are herbal teas, but lot of tea mixtures can be found on the markets based on special rulebook for herbal teas (33, 27).

Conclusion

Although the total number of registered herbal medicines and traditional herbal medicines in the Republic of Serbia is modest in comparison to most of the EU country members, the legal procedures for registration of these products are the same. The application of registered HMs and THMs constitutes a modern phytotherapeutic approach and a part of pharmacotherapy that has yet to find its place in evidence-based medicine.

Acknowledgement

This work is supported by the Ministry of Education Science and Technological Development of the Republic of Serbia (451-03-68/2022-14/ 200113) and by the Internal Project (No. 15) of the Faculty of Medicine, University of Niš.

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Pregledni rad **UDC: 615.322:582(497.11)**
doi:10.5633/amm.2022.0411

BILJNI I TRADICIONALNI BILJNI LEKOVI U REPUBLICI SRBIJI

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Prema Zakonu o lekovima i medicinskim sredstvima Republike Srbije, koji je usklađen sa evropskim direktivama o lekovima, supstanca koja predstavlja aktivnu komponentu leka može biti i biljnog porekla. Biljni lekoviti proizvodi (biljni lekovi – BL i tradicionalni biljni lekovi – TBL) primenjuju se za prevenciju i lečenje određenih oboljenja i stanja, njihovih početnih, blažih, ali i hroničnih i rekurentnih oblika.

U smernicama o farmaceutskom kvalitetu BL i TBL insistira se na rigoroznom i detaljnom definisanju polaznih biljnih sirovina, proizvodnog procesa i gotovih farmaceutskih proizvoda. Registracija ovih vrsta lekova obavlja se u Agenciji za lekove i medicinska sredstva Srbije. Tokom procesa registracije BL primenjuje se „Herbal Anatomical Therapeutic Chemical" sistem koji je je sastavni deo WHODrug Global sistema za klasifikaciju proizvoda i supstanci (HATC). Za sada je broj registrovanih BL i TBL u Srbiji skroman, u poređenju sa njihovim brojem u većini zemalja članica Evropske Unije. Agencija za lekove i medicinska sredstva Srbije kontroliše uputstva za lekove u kojima moraju biti naznačeni, između ostalog, i svi podaci o indikacijama, doziranju, kontraindikacijama, merama opreza, neželjenim reakcijama i interakcijama. Dakle, primena registrovanih BL i TBL predstavlja savremeni fitoterapijski pristup i najbezbedniji način korišćenja biljnih lekovitih supstanci i preparata.

Pored lekovitih, na tržištu postoje i druge kategorije biljnih proizvoda (biljni dodaci ishrani, biljni čajevi itd.), koji nisu namenjeni terapiji i lečenju. Stavljanje u promet dijetetskih proizvoda zasniva se na drugim zakonima i pravilnicima, a može se realizovati posle upisa u Registar dijetetskih proizvoda, za šta je nadležno ministarstvo za poslove zdravstva.

Acta Medica Medianae 2022;61(4):77-86.

Ključne reči: *biljni lekovi, tradicionalni biljni lekovi, tržište Republike Srbije*

Review article

UDC: 616.61-008:616.12-091
doi:10.5633/amm.2022.0412

THE EMERGING BENEFITS OF RENALASE BASED ON PRECLINICAL STUDIES: THE CURRENT PERSPECTIVE

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The Mammalian Gene Collection Project enabled the discovery of a novel kidney enzyme, subsequently named renalase in 2005. Renalase was initially identified in proximal renal tubules, however the following research reveals its broad pattern of tissue expression. Evidence demonstrates its cytoprotective properties, establishing it as a survival element in various organ injuries (heart, kidney, liver, intestines), and as a significant anti-fibrotic factor, owing to its, in vitro and in vivo demonstrated pleiotropy to alleviate inflammation, oxidative stress, apoptosis, necrosis, and fibrotic responses. Effective anti-fibrotic therapy may seek to exploit renalase's compound effects such as: lessening of the inflammatory cell infiltrate (neutrophils and macrophages), and macrophage polarization (M1 to M2), a decrease in the proinflammatory cytokines/chemokines/reactive species/growth factor release (TNF-α, IL-6, MCP-1, MIP-2, ROS, TGF-β1), an increase in anti-apoptotic factors (Bcl2), and prevention of caspase activation, inflammasome silencing, sirtuins activation, and mitochondrial protection, suppression of epithelial to mesenchymal transition, a decrease in the pro-fibrotic markers expression (α-SMA, collagen I, and III, TIMP-1, and fibronectin), and interference with MAPKs signaling network, most likely as a coordinator of pro-fibrotic signals. Mounting studies set the stage for renalase's pleiotropy to the level of cancer, particularly as a molecular driver for specific cancers, such as pancreatic, melanoma, renal, and breast cancer. The observation of renalase's enzymatic activities, particularly its interference with catecholamines metabolism, and regulation of plasmatic concentration, initially lead to the conclusion that renalase may significantly affect blood pressure regulation.

This review provides the scientific rationale for renalase's scrutiny regarding various organ injuries, and there is great anticipation that these newly identified pathways are set to progress one-step further. Although substantial progress has been made, indicating renalase's therapeutic promise, more profound experimental work is required to resolve the accurate underlying mechanisms of renalase before any potential translation to clinical investigation.

Acta Medica Medianae 2022;61(4):87-96.

Key words: *renalase, heart fibrosis, kidney fibrosis, MAPKs, TAMs*

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Introduction

The Mammalian Gene Collection Project enabled the discovery of a novel kidney enzyme, subsequently named renalase (1, 2) in 2005. Renalase was initially identified in proximal renal tubules (3-5), however the following research reveals its broad pattern of tissue expression, which includes the heart (6-13), brain (14, 15), liver (16, 17), pancreas (18), intestines (19), skeletal muscles (20) and the eyes (21). Mounting studies that followed set the stage for renalase's pleiotropy to the level of cancer, particularly as a molecular driver for specific cancers, such as pancreatic (22, 23), melanoma (24, 25), renal (26) and breast cancer (27). However, the most intriguing of the emerging findings indicated some promising benefits regarding renalase's expression in the human placenta, from the earliest stages of its development, suggesting its relevant role in human growth and gestation (28). The observation of renalase's enzymatic activities, particularly its interference with catecholamines metabolism, and regulation of plasmatic concentration, initially lead to the conclusion that renalase may sig-

nificantly affect blood pressure regulation (29). Given its more recent behavior as a pro-survival agent, particularly in the event of various organ injuries, and its potential to lessen the extent of an acute injury, renalase was assessed as a potentially relevant therapy option for diverse pathologies (30-44).

This review comprehensively summarizes the most up-to-date of results of preclinical studies, indicating the discovery that renalase functions as a pleiotropic molecule that likely protects different organs (kidney, heart, liver, intestines) against ischemic and toxic injuries. It also provides insight into renalase's role as a survival factor for tumor cells, since we now know that dysregulation of renalase signaling enables the survival and growth of melanoma and pancreatic cancer cells.

Renalase's biology

Renalase, named for its discovery, has been initially identified as a flavin adenine dinucleotide (FAD)-dependent amine oxidase, synthesized and secreted by the kidneys (1, 2, 29), resulting in a plasmatic concentration of approximately 5 μg/mL. This flavoprotein has been determined to function as a monoamine oxidase (MAO)-C, showing that less than 14% of amino acids identity with MAO-A. However, up until now, renalase has been identified as only a monoaminoxidase found in the blood, that, when *in vitro*, degrades catecholamines (29). Abundant research evidences the lack of renalase in patients suffering from chronic kidney disease. This perception poses the hypothesis that renalase deficiency accounts for the significant catecholamine excess which has often been observed in chronic kidney disease, as well as subsequent cardiovascular complications. Moreover, by metabolizing catecholamines, renalase likely decreases blood pressure, cardiac contractility, and heart rate, and prevents the compensatory increase in peripheral vascular tone (2, 29). It is however acknowledged that renalase activity in the blood reflects the level of the sympathetic tone, while in the setting of brief peaks of catecholamine blood levels, the activity, secretion, and synthesis of renalase are up-regulated resulting in significant hemodynamic effects, particularly *in vivo* (29). It is entirely possible that catecholamines induce a conformational change in the prorenalase molecule, or it may indicate proteolytic cleavage of prorenalase results in the rapid activation of renalase (29). It is widely accepted that plasma catecholamines and sympathetic tone are permanently increased in patients with chronic kidney disease, even after successful renal transplantation. This phenomenon likely contributes to the pathophysiology of hypertension, left ventricular hypertrophy, and ultimately, cardiac failure. The results of the aforementioned research give rise to hope that renalase replacement therapy may be highly beneficial in patients who are suffering with kidney disease. Additional research recognizes renalase's health benefits, extending far beyond the kidneys (heart, liver, intestines, skeletal muscles) as aforementioned, whereas its wide range of relevance has posed important questions as to whether renalase provides any additional advantages, far beyond only the catalytic molecule.

An outstanding advancement in renalase's pathophysiology was made upon the discovery that this protein exerts potent cyto protection, independent of amine oxidase activity. Indeed, both *in vitro* and *in vivo*, it has been documented that renalase effectively protects against toxic injury, such as cisplatin- and hydrogen peroxide-induced necrosis, by activating the intracellular signaling network, functioning entirely separately from its catecholamines-metabolizing properties (4). The up-regulation of protein kinase B (PI3K/Akt), mitogen-activated kinases (MAPKs), and extracellular signal-regulated kinase 1 and 2 (ERK ½), as well as the down-regulation of c-Jun N-terminal kinases is evidenced to be critical. These findings promote renalase protection in the animal model of acute kidney injury (AKI) (4). This observation supports subsequent cross-linking research in resolving a potential receptor for extracellular renalase. Accordingly, the plasmatic membrane calcium ATPase 4b (PMCA4b) has been identified as the receptor for renalase that, following its activation, sets in motion a choir of various signals from within the cells, presumably in order to promote its protective properties. In line with these findings, additional results indicate that renalase, by targeting its receptor, activates numerous downstream signaling, including acting as a signal transmitter as well as the activator of transcription (STAT3), NOS, NF-κβ, c-AMP, Ca^{2+}, p38, and Ras/Raf/MEK/ERK (37, 45). In line with the aforementioned, there are some transcriptional factors established as regulators of renalase gene expression, including TNF-α, HIF-1α, NF-κβ, STAT3, Sp1, some of which are ironically related to inflammatory responses (43, 45, 46-50). Among these regulatory pathways of particular clinical relevance may be renalase's positive feedback loop with STAT3 (45), a recognition that may be further investigated in the field of cancer pathology. Accordingly, renalase's link with HIF-1α, in which renalase mediates the protective effects of HIF-1α, may be valuable in the settings of ischemia/reperfusion injuries (at least in the heart and the kidneys).

The newly discovered recognition that factors included in cell proliferation, apoptosis, inflammation and overall protection are very closely linked to renalase's pathophysiology presumably implies diverse and distinct renalase cell signalization pathways, providing this molecule a multifaceted function in tissue homeostasis and various organ protection.

Renalase and the kidney

A growing body of evidence for the pro-survival effects of renalase in the field of acute kidney injury nominates this protein for more comprehensive scrutiny regarding the resolution of acute injuries (51, 52). For instance, contrast-induced nephropathy, and the possible occurrence of chronic

kidney disease represents an ongoing concern in the field of invasive cardiology. Acknowledging that renalase performs a pivotal role in blood pressure regulation and cellular survival, renalase has been assigned the competence of being a potential biomarker for AKI, indicating the existing loss of renal function and specifying disease severity (52). Namely, lack of the renalase gene (44) in animals exposed to renal ischemia reperfusion injury leads to significant renal tubular necrosis, inflammation and apoptosis, while, in ischemic and toxic (cisplatin-induced) AKI recombinant renalase supplementation, these changes are alleviated (44). In accordance, renalase-knocked-out mice subjected to cisplatin develop an increase in their plasmatic creatinine levels, significantly improve their renal injury score, the degree of apoptosis, and infiltration of macrophages. *In vitro* (HK-2 cells) recombinant renalase administration protects the cells against cisplatin- and oxidative (hydrogen peroxide-induced damage) injury, and furthermore, delays ischemic damage (4). Pivotal conclusions of the aforementioned study are that renalase prevents AKI, independent of its amine oxidase activity, and that its intracellular signaling is enabled via the rapid increase in phosphorylation of extracellular signal-regulated kinase 1 and 2 (ERK), and p38 MAPK signaling. At the same time, it decreases the phosphorylation of C-Jun N-terminal kinases. Subsequently, it has been demonstrated that renalase exerts its protective effects through ERK1/2, p38, and PI3K/Akt signaling networks by activating its receptor which has been previously identified as PMCA4b (35, 37, 40), (see above). More recent evidence of renalase's effects in cisplatin-induced AKI provides another important feature of its protective mechanism (5). However, both *in vitro* and *in vivo* it has been established that renalase significantly interferes with mitochondrial dynamics, as well as sirtuin 3 (SIRT3) levels by suppressing mitochondrial fission and reactive oxygen species production (5). It is worth noting that sirtuin 3 represents one of the mitochondrial deacetylases, presumably protecting all aspects of mitochondrial metabolism and the homeostasis of multiple organs (53). Its dysfunction is accordingly associated with age-related diseases, such as cancer, heart disease and metabolic diseases, suggesting sirtuin 3 as an applicable therapeutic target. It may be hypothesized that renalase, by functioning in a sirtuin 3-dependent manner, may establish various organ protection, far beyond protecting only against cisplatin-induced AKI.

The protective effects of renalase in the animal model of renal ischemic/reperfusion injury underscores its role as a protector of the kidneys. Ischemic preconditioning, prior to ischemic kidney injury, lessens renal inflammatory response, thus alleviating the degree of tubular necrosis and oxidative stress, at least in part, by renalase up-regulation (54). These remarks provide the hypothesis that renal ischemic preconditioning protection is mediated by renalase, and that renalase up-regulation is achieved by activation of TNF-α/NF-$\kappa\beta$ signaling. Positive effects of this process such as amelioration of the renal function, attenuation of tubular injury, and reduction of ROS and inflammation were abolished by simply silencing the renalase gene (54). This research emphasizes the renoprotection effects of renalase administration against contrast-induced nephropathy, providing the hypothesis that renalase therapy may represent clinically administered, contrast-induced nephropathy prevention. Subsequent research serves to additionally confirm that renalase pre-treatment markedly preserves renal function, mitigates tubular necrosis, oxidative stress, apoptosis, and inflammation in animals exposed to contrast-induced nephropathy (3), providing another indicative support for renalase's anti-oxidative, anti-necrotic, and anti-inflammatory properties. In line with these findings, renalase exerts have confirmed *in vitro* protection against loversol-induced cytotoxicity, which significantly abolishes caspase-3 activity, reactivating oxygen species generation and H_2O_2-induced apoptosis, hinting at the pivotal mechanisms of renalase's cytoprotection, by suppressing oxidation, apoptosis and inflammation mechanisms (43).

As a result, renalase anti-fibrotic properties have been further established and confirmed, and two meaningful pathways are proposed (30, 31). It is suggested that renalase alleviates renal fibrosis by reducing the production of reactive oxygen species, and perhaps even more importantly by suppressing oxidative-stress-induced epithelial-mesenchymal transition (EMT). It has to be mentioned that the epithelial-mesenchymal transition (EMT) represents an evolutionary process whereby epithelial cells acquire mesenchymal fibroblast-like features, such as decreased intercellular adhesion and enhanced mobility, making it one of the essential wound healing processes (55). The sequence of actions such as wound healing, tissue regeneration, and organ fibrosis represents a reparative-associated process in response to chronic inflammation-induced fibroproliferation that eventually leads to organ fibrosis and failure. Accordingly, beyond MDA suppression and the restoration of SOD expression, the administration of renalase abolishes oxidative stress-induced α-SMA, fibronectin, and collagens (I and III), thus restoring E-cadherin expression (as a marker of epithelial cells), in a dose-dependent fashion (30), as well as restoring H_2O_2-mediated epithelial-mesenchymal transition and fibrosis *in vitro*. The other study, however, confirms the same anti-fibrotic effects of renalase, but provides another plausible mechanism of its protection, namely by inhibiting activation of ERK 1/2 signalization (31). This study, however, explores how renalase therapeutic effects in animals subjected to unilateral ureteral obstruction, and assesses the capacity of renalase to suppress the transforming growth factor-β1 (TGF-β1)-induced EMT in the culture of proximal renal tubular epithelial cells (HK-2). Renalase significantly mitigates the progression of interstitial fibrosis in kidneys, via EMT inhibition, whereas renalase's primary activity is reveled to be the inhibition of the ERK 1/2 pathway. This data, collectively, provides additional theoretical support that the administration of rena-

lase in chronic kidney disease patients may effectively serve to mitigate the disease's progression.

Renalase and the heart

The initially identified effects of renalase in kidney pathology were additionally confirmed in preclinical models of acute myocardial ischemia (42). The study revealed that the administration of recombinant renalase significantly reduced the size of the necrotic field, and that cardiac hypertrophy is lessened, due to renalase application. (42). Subsequent research demonstrates that renalase represents a target gene of hypoxia-inducible factor-1α (HIF-1α) (43). The same study however, identifies that renalase down-expression in the heart results in a greater significance of ischemic/reperfusion injury, increased size of necrosis and aids in the prevention of decreased ejection fraction (EF). The studies that follow, beyond confirming the pro-survival properties of renalase, suggest the mechanisms of its cyto-protection, which are most likely administered by reducing inflammation, apoptosis, and necrosis, and by suppressing fibrotic responses. It is initially confirmed that renalase protects the cardiomyocytes against ischemia and reperfusion injury by lessening the level of necrosis and apoptosis, (6), supposing renalase as a novel cardiovascular drug for ischemia/reperfusion injury. In the preclinical model of chronic kidney disease (rats were subjected to 5/6 nephrectomy), renalase administration significantly preserves cardiac phenotype, including left ventricular (LV) hypertrophy prevention, LV hydroxyproline concentration (as a measure of cardiac fibrosis) and LV papillary muscle dysfunction (7). Such promising results opens up the possibility for renalase utilization in cardio-renal pathology, particularly in patients with chronic kidney diseases who have developed cardiac hypertrophy. Similar study models (41) reveal that renalase attenuates the progression of cardio-renal syndrome, whereas the administration of recombinant renalase reduces proteinuria, glomerular hypertrophy, and renal interstitial fibrosis. These effects parallel the significant down-regulation of pro-fibrotic gene markers, pro-inflammatory cytokines (TNF-α, IL-6, and MCP-1) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase components, such as gp91^{phox}, p47^{phox}, and p67^{phox}. At the same time, cardio-protective properties of renalase are also evident, as well as hypertension alleviation, cardiac hypertrophy and interstitial fibrosis mitigation, as well as cardiac remodeling prevention via profibrotic genes down-regulation and decreased phosphorylation of ERK-1/2 (41). Moreover, the observation that renalase likely influences the activation and infiltration of macrophages, including their polarization toward the M2 (CD163) phenotype, and suppresses M1-like (CD68) cells, which confirms the proposed hypothesis of renalase functioning as an anti-inflammatory agent. Finally, cardiac fibrosis evaluation, determined by Masson staining, demonstrates that renalase-supplemented animals have been shown to have less matrix deposition and cardiac fibrosis and expression of TIMP-1 and TGF-β, whereas the expression of MMP-1 was upregulated in renalase-treated animals. In the latest research demonstrates that by measuring renalase expression in kidney biopsies in patients with diabetic nephropathy, and in mice with renalase deficiency, renalase exerts a significant protective outcome (38). As evidenced, mesangial hypertrophy, renal inflammation, and pathological injury in animals with diabetes mellitus were more significant in comparison with control mice, whereas in animals with renalase up-regulation, the renal injuries were attenuated. Renalase apparently mitigates high glucose-induced profibrotic gene expression and p21 expression by suppression of ERK½ signaling. Presumably owing to its reno-protective behavior, renalase may be used for amelioration of nephropathy in patients with diabetes mellitus. Similarly, by impediment of the same signaling network, ERK½, including p38, renalase promotes significant mitigation of pressure overload-induced heart failure occurring in rats (40). Such results benefit renalase as a possible left ventricular hypertrophy biomarker, implying its advantage as a potential option for heart failure therapy. Specific single nucleotide polymorphisms evidenced in the renalase gene have been most recently shown to be associated with increased risk for several diseases (56-58). In particular, plasma renalase is documented to be increased in patients presented with unstable angina pectoris and metabolic syndrome (56), whereas the renalase rs10887800 polymorphism implies a significant association with unstable angina and metabolic syndrome development. Furthermore, the perception that the renalase Glu37Asp polymorphism is associated with left ventricle hypertrophy in females with aortic stenosis, and likely alters the binding affinity of the hypoxia- and hypertrophy-related transcription factors, provides proof of the principle that renalase presumably has a role in in hypertrophic response (57). Another clinically relevant setting for renalase determination may be patients with acute coronary microvascular dysfunction (59), providing a role for renalase as a possible biomarker for ischemia. However, renalase demonstrates the ability to predict coronary microvascular dysfunction (CMD) after multivariable adjustments (Framingham risk score), indicating its elevation in response to ischemia from acute CMD, deeming it a possible biomarker for ischemia (59). The observation of renalase's multi-functionality has already been reviewed elsewhere (34, 35, 60), providing the framework for renalase's subsequent experimental research regarding its emerging potential in the modulation of the cardio-renalaxis. If proven that renalase constitutes a missing patho-physiological link in the interplay between the kidneys and the heart, and that it may be used as a relevant cardio-protective agent for patients suffering from chronic kidney disease, it will present an outstanding value for mitigation of cardiovascular disease in patients on dialysis as well as for patients after undergoing kidney transplantation.

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Renalase and gastrointestinal system

The protective effects of renalase in the aforementioned settings of acute kidney and heart injuries has served to nominate this pro-survival factor for a new introduction into the context of another acute injury, the murine model of acute pancreatitis (18). The research however demonstrated that cerulenin-induced acute pancreatitis was significantly mitigated, both *in vitro*, and *in vivo*, when recombinant renalase was administered (prophylactically or therapeutically) after the injury, and that renalase-knocked-out animals exerted a greater severe pancreas injury. This evidence implies that renalase presumably mediates inflammation, at least in part, by hindering the accumulation, activation, and polarization of macrophages (M1 to M2), and macrophage-dependent IL-6 secretion. Levels of renalase in plasma are significantly decreased at the onset of acute pancreas lesion, indicating renalase to be a diagnostic or predictive marker. The noteworthy observation of this research is that plasma renalase markedly increases, far above the basal levels, during the later stage of the injury, indicating renalase biology far beyond being simply a pro-survival factor. Indeed, renalase has been suggested as a factor included in tissue recovery, as well as a pro-fibrotic agent (61). The acknowledgement that renalase affects Ca^{2+} signaling, *via* activation of its receptor PMCA4b, as previously mentioned, and the hypothesis that renalase protects against acute pancreatitis by modulating Ca^{+} transport, provides proof of the principle that renalase administration may be a relevant approach for acute pancreatitis patients. Besides mitigating pancreatic injury, the protective effects of renalse are observed in the following study of oxidative liver damage (62), more precisely in the murine model of ischemia/reperfusion injury that was superimposed on fatty liver disease. Similarly, to the protective effects on pancreatic tissue, recombinant renalase administration preserves the liver phenotype (necrotic area, the level of apoptosis, and the serum con-centration of ALT, AST, and LDH) both *in vivo* and *in vitro*. The underlying mechanism of renalase's cyto-protection is likely the reduction of reactive oxygen species generation, and the amelioration of the mitochondrial function *via* SIRT1 activation. It is worth mentioning that sirutin 1 (SIRT1) represents a histone deacetylase, localized in the nucleus and cytosol, pertaining to the sirtuin family (1-7), a class of nicotinamide adenine dinucleotide (NAD+)-dependent enzymes with multiple metabolic and pro-survival functions (63). Sirtuin 1 is vastly included in the regulation of cell survival in response to different stimuli, is associated with lifespan, and is gradually reduced with the onset of human aging, whereas its deficiency likely promotes age-related diseases (63). In this particular research (62), evidence shows that the lack of renalase leads to the down-regulation of SIRT1 expression and activity, and that recombinant renalase application regulates the expression and activation of SIRT1. In line with these findings, NAD+ represents the essential substrate for sirtuins (including sirtuin 1), whereas it has already been acknowledged that renalase may oxidize α-NADH, thus converting it to β-NAD+. Such results imply that renalase, *via* NAD+ levels elevation, likely upgrades the expression and the activity of sirtuin 1. Nonetheless, these results promote renalase as a relevant approach for liver oxidative injury mitigation. If confirmed that renalase may upgrade the activities of sirtuins, as already evidenced with SIRT1 (62) and SIRT3 (5), this finding would open up an entirely new area of research for renalase scrutiny. Regarding further benefits in liver protection (16), it is reported that renalase is significantly up-regulated in liver tissues that have undergone the ischemia/reperfusion process, and that these increased levels may be effectively suppressed by anti-oxidant therapy (16). Such responsiveness to oxidative stress provides renalase the feasibility of becoming a marker for the assessment of liver ischemic injury, particularly in patients subjected to liver surgery. Finally, the expression of renalase is increased in the mice model measured by fasting-induced oxidative stress, followed by the activation of NF-κβ p65 (19), and is considered to be a mechanism of intestinal anti-oxidative protection. When administered together, the results of these studies, beyond indicating renalase as a pro-survival factor, imply the possibility that the environment, as well, may play a part in modulating the levels of renalase, initially in tissues, and subsequently in the plasma. Such observation further underscores the role of renalase in the setting of the acute injuries, and emphasizes the need for its expanded scrutiny.

Renalase and cancer

Albeit not extensively researched in cancer pathology, most up to date knowledge of renalase's potential roles in pancreatic cancer and malignant melanoma has raised the perception that it may be also used for additional research in oncology. Moreover, it may be hypothesized that renalase's pivotal mechanisms such as acting as a context-dependent interference with ERK1/2, PI3K/Akt, signal transducer and activator of the transcription 3 (STAT3) signaling network including a positive feedback loop with STAT3 (4, 45) may be exploited by cancer cells aiming towards their proliferation and survival. It is initially documented that several types of cancer express increased levels of renalase: these cancers include pancreatic, bladder, breast and melanoma (35). Moreover, the study of pancreatic ductal adenocarcinoma (23), reveals that renalase demonstrates a two-fold increase in diseased pancreatic tissue, in comparison to healthy pancreatic tissue, and, over an average three-year mortality, generally implies that renalase likely promotes tumor cell survival and growth. Additionally, *in vitro* research argues that renalase administration increases pancreatic cancer cells survival rate from twofold to fivefold (23, 35). The same study further confirms that the inhibition of renalase signaling by siRNA or by inhibitory antibodies lessens the viability of pancreatic cancer cells, and enhances the apoptosis and cycle

arrested interruption of tumorous cells (23, 35) Taken together, these findings indicate that renalase-mediated signaling in cancer, if up-regulated, plays a decisive role in the pathophysiology of pancreatic cell carcinoma, making way for the possibility that the inhibition of the renalase signals may represent the anticipation of pancreatic cancer therapy. Moreover, renalase likely exhibits the prognostic potential for pancreatic cancer, or potentially, as a surrogate marker for treatment response or disease recurrence (23, 35). In a more recent study (22), regarding the identical pathohistological type of tumor (pancreatic ductal adenocarcinoma), increased circulating renalase concentration is demonstrated to be increased in both, in both premalignant and malignant tissues, compared to normal pancreatic cells, and is associated with worsened tumor characteristics, including greater angiolymphatic invasion, and greater node positive disease. Accordingly, overall survival is evidenced to be worsened in patients with increased renalase levels. Plasma renalase also predicts whether patients with locally advanced/borderline resection able pancreatic carcinoma should undergo resection. Collectively, elevated levels of renalase in premalignant pancreatic tissue plasma is associated with the nature of advanced tumors, therefore, its plasma concentration correlates with the clinical presentation of the disease, with a decreased level of overall survival and with reduced resect ability for locally advanced/ borderline cancer patients (22). Taken together, renalase shows some degree of promise as a novel tissue and serological biomarker in pancreatic ductal adenocarcinoma, and may presumably guide therapies, including resect ability in cases of pancreatic cancer. Moreover, the expression of renalase suggests its potential role in tumor biology and pathophysiology, upholding the potential for therapies by inhibiting the pro-survival effects of renalase in pancreatic ductal adenocarcinoma.

In line with prior discussions, the renalase expression is further assessed in melanoma, a disease presented with significant dysregulation of the signaling pathways that have been indicated to be under the supervision of renalase (MAPK, PI3K/Akt and JAK/STAT). The study presented out-standing evidence that renalase expression progressively increases from healthy skin tissue, to benign nevi and primary malignant melanoma, and that it is significantly increased in metastatic melanoma (24, 35). Besides indicating increased levels in primary melanomas, its significant expression was detected in CD163+ (M2-like) tumor-associated macrophages. Furthermore, renalase tumor expression (in clinical specimens) inversely correlates with disease-specific survival, implying the particular role of renalase in the pathophysiology of malignant melanoma (24). Renalase inhibition by antibodies, such as derived monoclonal antibody m28, or a renalase-derived inhibitory peptide therapy, decreases melanoma cell survival, and studies show that anti-renalase therapy blocks tumor growth within in vivo experimental models. Within a pathophysiological context, tumor cells exhibit increased apoptosis related to p38 MAPK-mediated Bax activation, followed by increased expression of the cell-cycle inhibitor p21. Moreover, the receptor for renalase, PMCA4b, mediates renalase-dependent STAT3 and ERK1/2 phosphorylation in melanoma cells, whereas dysregulating renalase signaling likely induces the polarization of macrophages towards M2 subclass, which presumably promotes tumor progression. Overall, if dysregulation of renalase signaling promotes the survival of cancer cells, a therapeutic approach objecting to halt these signals, which would therefore inhibit tumor growth, may vastly contribute to the holistic management of melanoma (24). Accordingly, inhibition of renalase expression in immune and host cells is associated with tumor rejection in murine melanoma models, and when rechallenged by another administration of tumor cells fails to result in subsequent tumor development (25), as shown with mice subjected to the wild-type of melanoma, Tumor regression may be benefitted by renalase signal suppression, due to anti-renalase ensuing tumoricidal effects, by effectively tailoring the tumor microenvironment, emitting host-in-dependent, cytotoxic and growth inhibitory effects upon the tumor cells (25). Accordingly, mice lacking the renalase gene exhibit the regression of melanoma in a T-cell-dependent fashion. In line with these findings, the anti-renalase antibodies upgrade the activity of anti-PD-1 in two aggressive murine melanoma models that show poor responsiveness to PD-1 inhibitors, which have been shown to strongly endure the development of anti-renalase antibodies with PD-1 inhibitors, being a potentially effective therapy for melanoma which is resistant to anti-PD-1.

Regarding renalase expression in renal tumors, it is demonstrated that the chromophobe renal cell carcinoma and papillary renal cell carcinoma renalase tissue expression is significantly up-regulated in comparison to control group findings, strongly correlating with the Fuhrman grades of tumors (26). These perceptions nominate renalase as an applicable biomarker for the discrimination of renal cancer grades, whereas anti-renalase therapy seeks the possibility of future anti-cancerous potential. Moreover, beyond the observation that renalase widely exists in various mammary gland cells, its expression is demonstrated to be significantly higher in the estrogen receptor (ER)-positive breast cancer, compared with control tissue, and positively correlates with p-ERK1/2 expression (27). These observations imply the potential for renalase to become a novel biomarker for ER-positive breast cancer, and a potential therapeutic target for ER-positive/HER2-negative subtype cancer. ER expression, including the malignant cell proliferation and growth, may be obtained through the p-ERK1/2 pathway, as already demonstrated, as previously discussed.

Moreover, using immune-localization to resolve the particular types of tumorous cells that relate to renalase up-regulation, it is concluded that melanoma and tumor-associated macrophages significantly correlate with renalase expression (34). Collectively, this collective body of data supports the

hypothesis that molecule silencing and suppressing the effects of renalase for different types of cancer may be entirely effective as anti-cancer therapy, therefore these types of investigations should be highly encouraged and supported.

Conclusion

The results of the aforementioned research establish renalase as a relevant pro-survival agent in several injury settings, providing the potentially profound therapeutic utility of renalase-based therapy for acute tissue and organ injury (myocardial infarction, toxic and ischemic AKI, ischemic liver injury, acute pancreatitis). The data for renalase signaling inhibition, particularly regarding cancers, is also compelling. Conversely, another key goal may be to research the anti-renalase antibodies as a relevant therapeutic approach in treating cancer patients (pancreatic cancer and melanoma).

Even though, up until now, substantial progress on renalase biology has been made, proposed mechanisms regarding its uses, activities and effects calls for more intense scrutiny and a deeper understanding of its pathophysiology. This newly acquired knowledge, combined with the analysis of renalase signaling, will allow us the opportunity to create more effective therapy options, those which are eagerly awaited, and produce ongoing clinical settings for further exploration of renalase and its multi-functionality. Greater understanding of the complete pathophysiology of this somewhat enigmatic, however biologically powerful molecule, may lead to its broad clinical utilization, therefore, vast apprehensions exist regarding its future analysis and promising potential towards clinical research.

Acknowledgements

This research was supported by a grant from the Ministry of Science and Technological Development, project number 451-03-9/2021-14/200113, by a grant by Faculty of Medicine, University of Niš, INT-MFN-46, 2020-2023 and by the Project of Serbian Academy of Science O-06.17. The authors declare no conflicts of interest. This was an academic study, not supported by the industry.

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Pregledni rad **UDC: 616.61-008:616.12-091**
doi:10.5633/amm.2022.0412

POTENCIJALNA KORIST MERENJA RENALAZE U KLINIČKOJ PRAKSI – REZULTATI PRETKLINIČKIH STUDIJA

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Na osnovu rezulata dobijenih na projektu *Mammalian Gene Collection* 2005. godine otkriven je novi molekul, koji se sintetiše i sekretuje u tkivu bubrega, posledično nazvan *renalaza*. Ekspresija renalaze inicijalno je dokazana u proksimalnim tubulima, ali su dodatna istraživanja pokazala to da se u značajnoj meri može detektovati i u ostalim organima, primarno u tkivu miokarda, zatim u nervima, tkivima pankreasa, jetre i creva, skeletne muskulature i oka. Na osnovu rezulata nedavnih onkoloških studija, značajna ekspresija renalaze u velikom procentu postoji i u malignom tkivu pankreasa, dojke, bubrega i melanoma, uz hipotezu da ovaj flavoprotein potencijalno funkcioniše kao molekulski pokretač za pojedine karcinome. Rezultati novih istraživanja ukazuju na to da postoji visok stepen ekspresije renalaze u humanoj posteljici, od najranijih faza razvoja, sugerišući na njenu relevantnu ulogu tokom gestacije i razvoja ploda. Analiza enzimske aktivnosti renalaze, posebno njene uloge u katabolizmu kateholamina i održanju koncentracije u plazmi, implicira na potencijalnu ulogu u regulaciji krvnog pritiska i očuvanju kardiovaskularnog zdravlja. Pored enzimskog potencijala, renalaza se smatra i molekulom sa citokinskim efektima, naročito u slučajevima akutnih povreda. Na osnovu rezulatata dobijenih pomoću istraživanja na animalnim modelima akutnih oštećenja različitih organa (bubreg, srce, jetra), u kojima je dokazano da administracija renalaze može značajno da umanji stepen lezije tkiva i omogući preživljavanje, ovaj biološki potentan protein smatra se potencijalnom terapijskom opcijom za različite lezije.

Ovaj pregledni rad sumira i daje kritički osvrt na najnovije rezultate dobijene u pretkliničkim studijama, uz potenciranje plejotropije renalaze u zaštiti tkiva i organa (bubreg, srce, jetra, creva) od ishemijskih i toksičnih povreda. Dodatno je obrađena uloga renalaze kao faktora za preživljavanje tumorskih ćelija, s obzirom na to da je dokazano da disregulacija signalizacije renalaze omogućava preživljavanje i rast ćelija melanoma i raka pankreasa.

Acta Medica Medianae 2022;61(4):87-96.

Ključne reči: *renalaza, fibroza srca, fibroza bubrega, MAPK, tumorski makrofagi*

JEDINSTVENI KRITERIJUMI ZA OBJAVLJIVANJE NAUČNIH RADOVA U BIOMEDICINSKIM ČASOPISIMA

Ideja o postavljanju jedinstvenih kriterijuma za objavljivanje radova u časopisima za biomedicinske nauke iskristalisana je 1978. godine u Vankuveru. Ovi kriterijumi za rukopise, uključujući pravila za pisanje bibliografije, prvi put su objavljeni 1979. godine. Vankuverska grupa je vremenom prerasla u Međunarodni komitet urednika medicinskih časopisa — International Committee of Medical Journal Editors (ICMJE). Trenutno je na snazi peta revizija kriterijuma za objavljivanje radova u biomedicinskim časopisima, doneta 1997. godine.

Kriterijumi za citiranje i navođenje referenci

Reference se obeležavaju arapskim brojevima u zagradama, pri čemu se reference obele-žavaju brojevima onim redosledom kojim se pojavljuju u tekstu. Reference citirane jedino u tabelama ili legen-di moraju se obeležiti brojem u skladu sa redosledom pojavljivanja u tekstu.

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Izbegavati upotrebu apstrakata kao referenci, već koristiti samo izvorne tekstove (*in extenso* članci). Reference koje se odnose na radove koji su prihvaćeni, ali još nisu odštampani, treba označiti sa "u štampi", pri čemu autor mora imati pismeno odobrenje da citira takve radove i da priloži pismeni dokaz da je citirani rad prihvaćen za štampu. Informacije iz rukopisa koji nisu prihvaćeni za štampanje mogu se citirati u tekstu kao "neobjavljeni rezultati", ali sa pismenom dozvolom autora.

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Navesti prvih šest autora, ukoliko ih je više iza šestog dodati **et al.** ukoliko je referenca na engleskom jeziku ili **i sar.** ukoliko je referenca na srpskom jeziku.

Vega KJ, Pina I, Krevsky B. Heart transplantation is associated with an increased risk for pancreatobiliary disease. Ann Intern Med 1996; 124(11):980-3.

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Službeni izveštaji (Issued by funding / sponsoring agency):

Smith P, Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX): Dept. of Health and Human Services (US), Office of Evaluation and Inspections; 1994 Oct. Report No.: HHSIGOEI69200860.

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Kaplan SJ. Post-hospital home health care: the elderly's access and utilization [dissertation]. St. Louis (MO): Washington Univ.; 1995.

Druge vrste publikovanog materijala

Neobjavljeni materijal

19. U štampi (In press)

Leshner AI. Molecular mechanisms of cocaine addiction. N Engl J Med. In press 1996.

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20. Internet članak u elektronskom formatu

Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar "cited 1996 Jun 5"; 1(1)(24 screens). Available from: URL: http://www.cdc.gov/ncidod/EID/eid.htm

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