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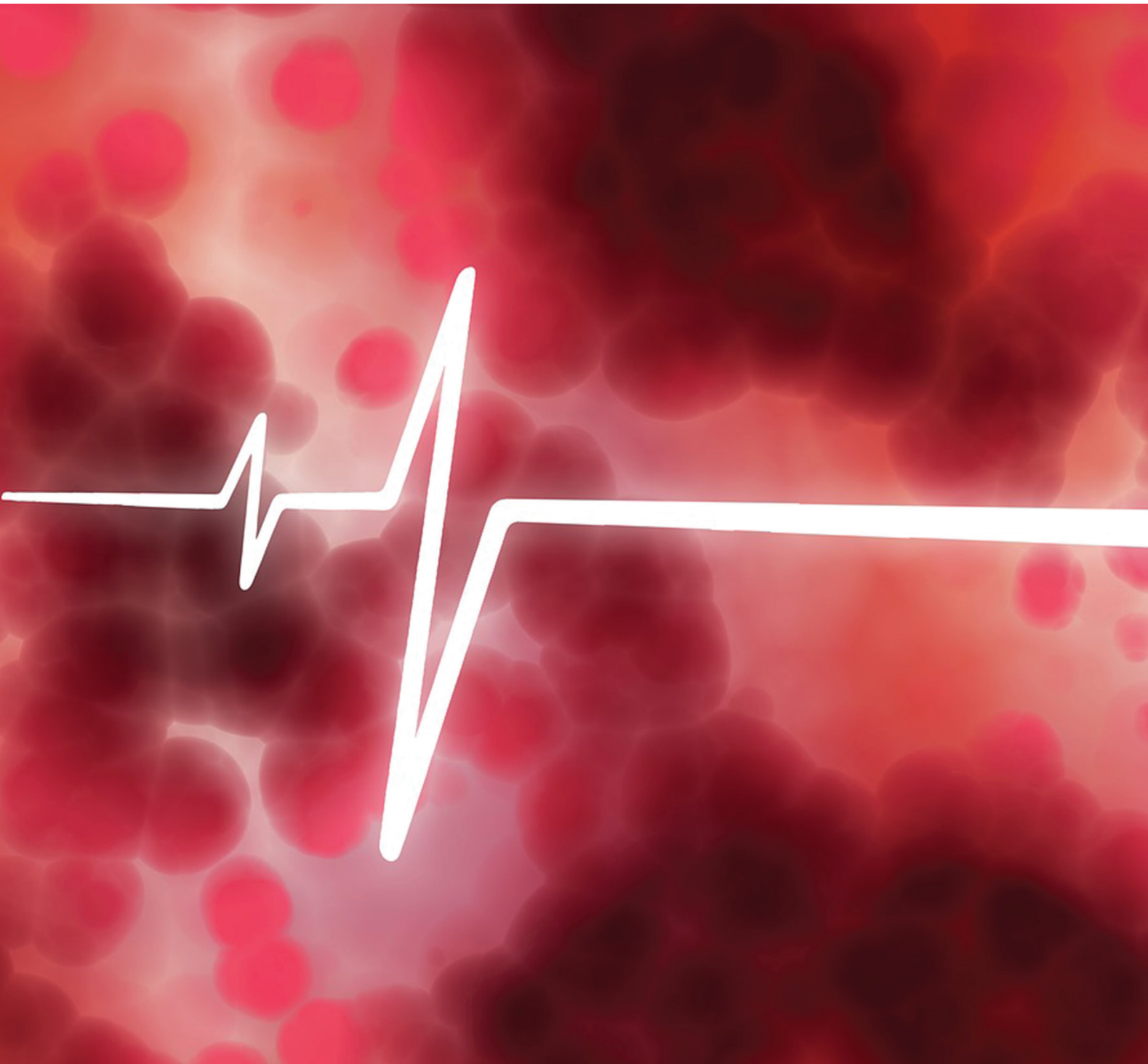

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Opinion Article

IN PULMONARY EMBOLISM AN INTERPRETATION OF SYSTOLIC BLOOD PRESSURE, TROPONIN (AND OTHER IMPORTANT PARAMETERS) DEPENDS ON THEIR PREVIOUS VALUES AND COMORBIDITIES

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Abstract. *We reviewed several important parameters in pulmonary thromboembolism (PTE) and showed how not only absolute values but also relative are relevant in clinical practice. The vast majority of parameters depend both on previous values and co-morbidities; failure to realize this can result in misclassification of a patient and inappropriate treatment. For example, the absolute value of systolic blood pressure (BP) less than 90 mmHg is crucial for urgent treatment (e.g. thrombolysis); obviously, the same admission systolic BP (sBP) of 87 mmHg may not have the same significance if previous usual sBP was also 87 mmHg or it was 220 mmHg. Moreover, cardiac troponin is also very important for the risk stratification; the same troponin concentration ought not to be interpreted equally if it is due to acute pulmonary thromboembolism or if it is chronic and due to e.g. renal failure. The interpretation of important dichotomous parameters (normal or pathologic values) in PTE does depend on previous values (if available) and co-morbidities. This principle should be recognized and used in clinical practice, while risk-stratifying patients.*

Key words: *Pulmonary thromboembolism, blood pressure, troponin, right ventricle strain, d-dimer.*

In addition to the patient's data, medical knowledge, and common sense, at least three aspects are certain for the evaluation of an individual patient: 1) in real life, the situation is far more complex (as opposed to books and guidelines); 2) the result of every single measurement is dynamic (in time), and 3) as a rule, the result of the measurement depends (sometimes dominantly) on the related factors.

Let us start with one of the key measurements in pulmonary thromboembolism (PTE): *the **blood pressure*** (*BP*). In addition to ongoing/very recent resuscitation, low systolic BP (sBP) (<90 mmHg) -with or without shock- is crucial in PTE to classify the patient as having a high risk with probable consequent administration of a thrombolytic (or proceeding to percutaneous/ surgical intervention) [1]. For the borderline sBP it is difficult to decide if it represents hypotension or not and if PTE is its single cause. Some of the reasons for this difficulty are: sBP is frequently unequal on left versus right arm and it depends on the proper measurement (how adequate dimensions of the cuff are and how tight it is placed, etc.). Moreover, sBP can change e.g. for ± 5 mmHg in a couple of minutes. Additionally, for a patient with PTE with sBP 87 mmHg on presentation, the usual sBP for months before the hospitalization may have been A) 87 mmHg (then 87 mmHg on admission is the patient's typical value, without any BP drop due to PTE) or B) 180 mmHg (then 87 mmHg on admission is approximately half of the usual value; it is likely profound hypotension (possibly PTE-induced) and it raises suspicion on shock) (Figure 1).

Shock is easier to recognize as compared to hypotension, and hypotension itself, even without shock is a marker of high risk in PTE and consecutively it speaks in favour of thrombolytic treatment. What we suggest is what is considered in everyday practice: *to include a relative aspect in the interpretation of the absolute values.*

Similarly, the correct classification of non-high risk PTE patients requires ***troponin***. Troponin is very important, but far from specific for PTE – apart from the crucial cause (acute myocardial infarction), the increased troponin concentration may be a consequence of

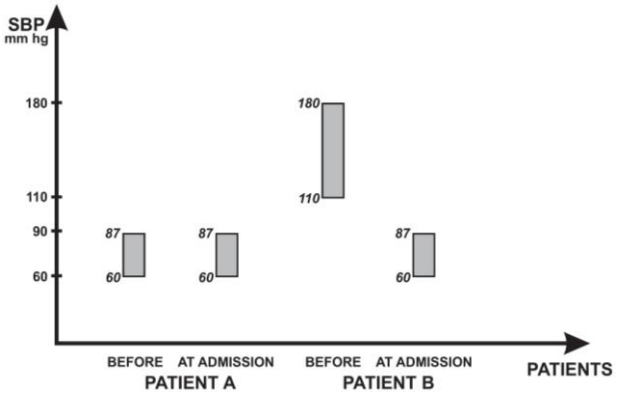

Fig. 1 SBP systolic blood pressure

numerous diseases, such as stroke, subarachnoid hemor-

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rhage, chronic kidney disease, infectious disease, etc [2]. Many of the causes of elevated troponin are chronic, for example, chronic renal failure (CRF) [2, 3]. As many as 33–43% of patients with CRF may have increased troponin concentration [4].

If a patient with CRF and chronic troponin elevation experience an acute venous thromboembolism (VTE), his/her troponin elevation may be misinterpreted as a result of this acute VTE event which could lead to misclassification. Therefore, if the cause of increased troponin in a patient with PTE is concomitant chronic heart or renal failure, it may be misleading for a physician to classify the patient as having intermediate-high risk PTE, according to the latest 2019 European Society of Cardiology (ESC) PTE guidelines [1, 2]. In order to avoid such fault we may make it more precise by stating: "increased cardiac troponin concentration in plasma, provided it is not due to other causes, such a renal failure". This would be completely in line with the statement about hypotension in the recent ESC guidelines for acute PE, where the experts exclude other prevalent causes of low BP aiming to underline the importance of hypotension which results from PE itself [1].

The similar situation is also true for N terminal-pro brain natriuretic peptide ***(NT-proBNP) / BNP***. Many non-cardiac conditions are capable of increasing their concentrations, such as advancing age, anemia, renal failure, obstructive sleep apnea, severe pneumonia, etc [5]. As with troponin, NT-proBNP / BNP elevation may be due to some of the other mentioned causes and present before the actual VTE event carrying the opportunity to be inadequately interpreted as caused by PTE; such misinterpretation could lead to higher patient's risk perceived by physician. Therefore, abnormalities of numerous parameters, which are used for the risk stratification of PTE, have the additional potential causes and can be chronic. The interpretation of a few other parameters also depends on previous values and comorbidities. For example, ***heart rate* (*HR*)** ≥ 110 beats per minute (bpm) is an important part of the original and simplified Pulmonary Embolism Severity Index (PESI) and it carries the most points (+30) following the altered mental status (+60 points) [1]. HR depends on the rhythm: in atrial fibrillation (AF), HR is usually higher. Therefore, it is easier for AF patients to "achieve" the HR ≥ 110 bpm with the same severity of PTE. This is a very good example of the influence of chronic disorder (co-morbidity) upon a parameter used for risk stratification.

Similarly, interpretation of the imaging parameters of the ***right ventricle (RV) strain*** (obtained by echocardiography and/or by computed tomography pulmonary angiography, *CTPA*) is also determined by the previous measures (and is frequently influenced by the co-occurrence of pulmonary and heart disease). ***D-dimer*** may also be increased due to numerous causes, such as bleeding, thrombosis [venous, including VTE and risk factors for it; arterial e.g., AMI, stroke, peripheral artery disease; microvascular thrombosis and intravascular thrombosis (disseminated intravascular coagulation – DIC, thrombosis due to foreign material, such as catheters, pacemakers, artificial valves); aortic dissection, other cardiologic diseases (e.g., AF, left ventricle aneurysm, heart failure, thrombus in the heart) as well as kidney and hepatic diseases and false positives] [6].

Therefore, the vast majority (if not all) parameters in PTE depend both on previous values and comorbidities; failure to realize this can result in misclassification of a patient and inappropriate treatment. Indeed, there is a positive example of "putting in the context" of previous status and comorbidities: it is stated in guidelines that *"*obstructive shock (systolic BP < 90 mmHg or vasopressors required to achieve a BP ≥ 90 mmHg *despite an adequate filling status*…*)"* is mandatory for one of the high-risk PTE varieties [1]. It is clearly stated that in a PTE patient hypotension is not always due to PTE only, but it may be a consequence of comorbidities (e.g., presenting with hypovolemia), or result from a combined origin.

Furthermore, for all 6 mentioned clinical scenarios (regarding sBP, HR, RV strain by echocardiography or CTPA, troponin, D-dimer, NT-proBNP / BNP) and in the given positive example (of "putting in the context") it is important to realize another point. An alternative cause (other than PTE) of the abnormal result does not necessarily imply a better outcome. For example, elevated troponin concentration is sometimes due to other diseases (e.g., chronic renal failure), not PTE itself, but irrespectively of the underlying disease, increased troponin value as the rule is a marker of worse outcome [1, 7]. Indeed, the presence of the other causes of the pathologic result suggests that PTE itself may not be a single problem and therefore, that other treatments should be used (not only for PTE). For example, if sepsis is diagnosed in addition to PTE, therapy should also include antibiotics together with PTE treatment, and sepsis may be the contributing/main cause of hypotension [1]. Moreover, if one finds bleeding in a patient with PTE and hypotension, it will also influence treatment choices. None of the other examples we described is elaborated or even mentioned in the excellent guidelines on the topic [1].

Therefore, all afore-mentioned 6 parameters are important in PTE, and their abnormalities are clinically relevant whatever the cause is (PTE or another one). Consequently, for proper clinical decisions, it is important to recognize if the change is acute and due to PTE or it is chronic and/or it results from other diseases.

Final Remarks

We do not disagree with guidelines' cutoffs; moreover, recommendations in guidelines ought to be followed, as they are the best we have contemporary for the vast majority of patients. The improvement we suggest is to take into consideration the individual characteristics during the previous period as well as co-morbidities (which influence directly some of the important parameters for PTE risk stratification). In this way we evaluate not only the abso-

lute value of a parameter but also if it is changed due to PTE itself or due to co-morbidity or if this abnormality is present from an earlier period. Therefore, the suggestion we advocate may serve to improve interpretation of particular parameters' interpretation, which may in turn improve the validity of the whole score and individual risk estimation. In everyday practice we do it often for numerous diseases: for example, we compare previous findings to the actual ones (such as ECG), to understand if there is a change and if it is recent or long-standing one.

The point we make is the importance of relative values in addition to using the absolute values only. There is no reason to waive relative values of important parameters. An illustrative example is low oxygen saturation (SpO_2): if it becomes low (e.g. 88% on room air) at the time of VTE then it is a sign of increased risk, while it may be the average SpO_2 for a patient with Chronic obstructive pulmonary disease who experience an acute subsegmental PTE.

Conclusion

The interpretation of important dichotomous parameters (normal or pathologic values) in PTE does depend on previous values (if available) and comorbidities. This principle should be recognized and used in clinical practice, while risk-stratifying patients. Moreover, this obvious principle probably deserves mentioning in the guidelines (having in mind how important they are).

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Original Scientific Article

SARS-COV-2 ANTIGEN SCREENING DURING THE FIRST POST-LOCKDOWN MAJOR MUSIC FESTIVAL IN NOVI SAD, SERBIA, IN JULY 2021

Maja Lazović[1], Veselin Bojat[2], Vladimir Vuković[1,3], Nina Smiljanić[2], Marija Milić[4], Radmila Velicki[1,3], Nataša Nikolić[1,3], Tatjana Pustahija[1,3], Mioljub Ristić[1,3]

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Abstract. *Many countries have resumed mass gathering events like music festivals, despite potential risk of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmissions. This study aimed to examine the frequency of SARS-CoV-2 infection among visitors to a mass event, the EXIT festival, in relation to the proposed preventive measures. A total of 466 visitors were included in this longitudinal study conducted in Novi Sad, Serbia, during July, 2021. All subjects were tested with RDT-Ag test for the presence of SARS-CoV-2 virus at the beginning of the study and seven days after. Basic socio-demographic and epidemiological data were collected through a questionnaire. The average age of participants was 28.34 ± 8.87 years with the majority of men (53.7%). There were 170 (36.5%) vaccinated participants. A 97.1% in vaccinated and 92.2% in unvaccinated group, reported to regularly wear a protective mask (p=0.029). At the second cross-sectional testing, 354 subjects were tested, of which 150 (42.4%) were vaccinated. There was no statistically significant difference in application of preventive measures during and after the festival, in respect to vaccination status of the participants. Twenty participants (5.6%) reported some of the COVID-19-like symptoms in the first seven days after visiting the festival, but no COVID-19 infection was confirmed at the RDT-Ag testing. Despite the potential effectiveness of applied preventive measures such as RDT-Ag screening, mask-wearing and vaccination, additional caution is needed when holding these events during a period of high SARS-CoV-2 transmission, as well as when the new virus variants emerge in community*.

Key words: *COVID-19, preventive measures, RDT-Ag testing, mass event, EXIT festival.*

Introduction

Mass gatherings represent an assembling of a larger number of people at a certain location, in a certain period of time and for a certain purpose, and as such are associated with an increased risk for the health of participants due to various factors [1,2]. During the COVID-19 pandemic, mass gatherings posed a significant public health challenge given the close contact between visitors, bringing to the increased risk of the SARS-CoV-2 virus transmitting to the susceptible population [3–5]. A recent review of the available literature showed that the most important factors associated with the risk of SARS-CoV-2 transmission are a longer duration of the mass event, organization of the indoor events and non-compliance with the preventive measures, while on the other hand, there was no correlation between the size of the event and the higher risk of SARS-CoV-2 transmission [5–7].

Governments of most countries in the world developed different strategies for organizing mass gatherings based on two fundamental approaches: cancelling or limiting mass gatherings, or limiting the number of visitors to these events [3,4]. The cancellation of most mass events in the first year of the COVID-19 pandemic had a huge impact not only on social aspects of human functioning but also on the economy of many countries [8]. The availability of COVID-19 vaccines in combination with other public health measures (e.g., mask wearing, physical distancing, disinfection) and better knowledge of the duration of immunity after COVID-19 infection have significantly changed the situation in many countries and indicated that SARS-CoV-2 transmission in the population can be controlled [9–14]. Thanks to this growing knowledge and the daily increase in coverage of vaccination against COVID-19, mass gatherings were allowed in many countries around the world [9,10].

The World Health Organization continues to underline the importance of mass gatherings (sports events, concerts, celebrations, etc.) on the intensity of transmission of COVID-19, especially in the vulnerable population in which there is limited immunity to this infection [15]. However, in countries where the epidemiological situation allows reopening and mass gatherings to occur, after a period of restrictions on movement, it is crucial to ensure that all decisions on the organization of mass events are based on the risk assessment of the intensity of virus transmission in population and include immuniza-

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tion in compliance with the adequate preventive measures [15]. Of concern is the fact that a large percentage of population in many countries has not been vaccinated yet. In addition, new variants of SARS-CoV-2 are emerging, which are more contagious than the previous ones and against which somewhat lower effectiveness of several vaccines has been observed [9,10,16,17]. As asymptomatic individuals and patients with a mild clinical presentation of COVID-19 significantly contribute to the transmission of SARS-CoV-2 in the community, screening testing of unvaccinated individuals, regardless of signs or symptoms of COVID-19, is an important approach to prevent and control the outbreak of COVID-19 in the population [11]. The use of RDT-Ag (rapid diagnostic test for detection of SARS-CoV-2 antigen) as the screening strategy is a suitable measure because of the lower testing costs and quickly available results, as well as the high specificity and some lower sensitivity of these tests in the early stages of SARS-CoV-2 infection when the infected person is the most contagious [11]. The use of a RDT-Ag as a screening test as part of a prevention strategy is important because of the short time required to obtain results, high specificity and sensitivity in the early stages of infection when viral load is at the highest, reduced sensitivity as the disease progresses and SARS-CoV-2 transmission becomes lower [11,18].

A number of studies investigating the effectiveness of comprehensive preventive measures during mass gatherings, such as the vaccination, wearing protective face masks, systematic RDT-Ag test screening, adequate ventilation of the indoor area and appropriate hygiene, have indicated the safety of mass gatherings and low risk of SARS-CoV-2 transmission, only if all prescribed preventive measures against COVID-19 are implemented [9,19–21]. Therefore, we the aim of our study was to examine the frequency of SARS-CoV-2 infection in a representative sample of visitors to a mass event, the EXIT music festival in relation to the applied preventive measures.

Material and Methods

Study Design

This longitudinal study was conducted in the period from July 8, 2021 to July 17, 2021 in the City of Novi Sad, Serbia, where the data of repeated measurements from the same sample of participants in two time periods, were collected. The study included visitors of the EXIT music festival, which is traditionally held outdoor, every year during July, at the Petrovaradin Fortress in Novi Sad, Serbia. The necessary sample size was calculated using the G-power program v. 3.1.6 [22] considering an alpha error of 5%, study strength of 0.80, a 95% confidence interval, and an average number of the EXIT festival visitors, which was around 50,000 people annually, in the previous 5 years. Thus, the calculated necessary sample size was 382 participants, but due to the probability of the lost to follow-up, the sample size was increased by 20%, so that at the beginning of the study a total of 466 visitors were included.

Special preventive measures were active at the festival site. Entry to the festival was allowed only for visitors who met at least one of the following conditions: 1. A proof of complete vaccination schedule, i.e. received two doses of any of the available vaccines against COVID-19, and more than 14 days have passed since the second dose; 2. A certificate of the previous COVID-19 in the last six months before the festival visit with a positive specific SARS-COV2 IgG antibody test; or 3. A negative RT-PCR (reverse transcription polymerase chain reaction) or RDT-Ag, not older than 48 hours before the visit. The same conditions applied to the visitors and organizers of the festival. Also, body temperature was measured before entering the festival, and hand sanitizers were available at several points at the festival site.

For the purpose of investigating the effectiveness of applied preventive measures, all visitors were monitored for seven days. The first cross-sectional testing was conducted at the beginning of the study, during the days of the festival, on July 8, 9 and 10, 2021. Basic socio-demographic data (gender, age, level of education, marital status) and epidemiological data (travel abroad in the past 14 days, presence of COVID-19 symptoms, contact with a confirmed case of COVID-19, previous COVID-19 infection and date of illness, presence of chronic diseases, vaccination status and type of COVID-19 vaccine received, adequate use of a protective face mask, and adherence to other prevention measures) were obtained through a specially designed questionnaire. The second cross-sectional testing was conducted one week after the first, i.e. on July 15, 16 and 17, 2021. The questionnaire provided additional epidemiological data on the potential risk of exposure to COVID-19 in the days after the festival (occurrence of COVID-19 symptoms in the days after the festival, contact with a confirmed case of COVID-19 in the days after the festival visit, regularity and duration of the use of a protective face mask in the days after visiting the festival, regular use of a protective face mask during the festival, staying in indoor space with a large number of people in the days after visiting the festival, adherence to other preventive measures in the days after visiting the festival, and the time spent at the EXIT festival).

Laboratory Methods

All enrolled subjects were tested with the RDT-Ag (STANDARD Q COVID-19 Ag Test, SD Biosensor, Gyeonggi-do, South Korea) [18] for the presence of SARS-CoV-2 virus in a sample of throat and nose swabs at the beginning of the study and seven days after the first test, regardless of their vaccination status. The seventh day is set for retesting because it represents the period in which laboratory confirmation can be obtained in about 95% of those infected with SARS-CoV-2 virus, when virus excretion is greatest and when the possibility of virus detection by antigen test is the greatest [23].

During the first visit to the EXIT festival, nasopharyngeal swab samples were collected by the trained healthcare workers from the Health Center Novi Sad together with the supervision of the study investigators, before entering the festival, and an RDT-Ag test was performed immediately at the sampling site. Respondents who were tested positive on the first test were excluded from the current study, isolated in the red area, and referred to the COVID-19 respiratory center of the Health Center Novi Sad for further diagnostics and treatment, in accordance with the current professional and methodological instructions of the Institute of Public Health of Serbia.

Study participants with a negative first RDT-Ag were invited by e-mail or telephone to the second test, seven days apart, when testing was performed with the same type of RDT-Ag test, at the Health Center Novi Sad.

Statistical Analysis

Descriptive statistics with the mean and standard deviation (SD) for continuous variables and absolute frequencies and percentage (%) for categorical variables were used for the data presentation. The Kruskal-Wallis test, Mann Whitney - U test, and Chi-squared test were used to examine the differences between continuous and categorical variables, where appropriate. The level of statistical significance was set at $p<0.05$. Data were analyzed using the SPSS Windows user package, version 19.

Ethical Considerations

The Ethics Committee of the Health Center Novi Sad approved the study protocol under the number 21/14-1, from July 7, 2021. The approval was also obtained from the director of the Health Center Novi Sad (decision number 21/14-2, from July 8, 2021). All visitors who agreed to participate in the study, and who met one or more inclusion criteria for this research study were informed about the purpose and the aim of the study, and gave their written consent to participate. Each participant was assigned with a special study code which was then entered inthe questionnaire used in the research as well as on the samples taken, in order to make the research process anonymous. Only the main study investigators knew the personal data of the respondents. All data were thus fully anonymized before the analyses were conducted.

Results

In the first cross-sectional testing, a positive RDT-Ag was obtained from two participants who were then excluded from the study monitoring, thus, a total of 466 subjects were included in the study, of whom 170 (36.5%) were vaccinated and 296 (63.5%) were unvaccinated (Table 1).

Out of 464 participants that gave information on sex, the majority (53.7%) were male. The average age of the respondents tested on the first day of arrival at the EXIT festival was 28.34 ± 8.87 years, where the youngest respondent was 15 and the oldest was 58 years old. Of 170 participants that were vaccinated, a 48.8% received Pfizer-BioNTech BNT162b2 vaccine, 30.6% received Sinopharm BBIBP-CorV, 11.2% Oxford-AstraZeneca ChAdOk1 nCoV-19, 8.2% Gamaleya Research Institute Gam-COVID-Vac, 0.6% were vaccinated with the Moderna mRNA-1273, while the one remaining participant (0.6%) did not provide data on the vaccine type. The vaccinated participants were on average 5.3 years older (mean = 31.70, SD=10.54 years) in respect to unvaccinated participants (mean = 26.41, SD = 7.08 years) ($p = 0.001$). The majority (44.1%) of vaccinated participants belonged to the 21-30 age group followed by 31-40 (18.2%) and 41-50 (18.2%) group, while in the unvaccinated group, the majority (52.7%) were in 21-30 years group, followed by the youngest group, 15-20 (23%) ($p < 0.001$). A total of 50.2% of the participants were single, with 42.9% in vaccinated group and 54.4% in unvaccinated ($p<0.001$). Regarding the level of formal education, majority (47.1%) in the vaccinated group had primary/secondary school education followed by participants with a university degree (40.6%) while in the unvaccinated participants inversion was noted and the majority (45.9%) had university degree followed by 42.9% of participants with primary/secondary level education ($p = 0.533$). Around 7% of vaccinated participants reported having some chronic disease with respect to just 2% in the unvaccinated group ($p=0.007$). A total of 9.4% of vaccinated participants reported travelling abroad in respect to just 3% of unvaccinated ($p=0.004$). Also, significantly higher percent (27.1%) of vaccinated participants reported having had a COVID-19 in the past in comparison to just 18.2% of unvaccinated participants ($p=0.026$). The majority of participants reported wearing a protective mask adequately, i.e. 97.1% and 92.2% in vaccinated and unvaccinated group, respectively ($p = 0.024$). High percent of participants (above 97%) in both groups reported adherence to other preventive measures against the spread of COVID-19. Sex of the participants, level of education, contact with a person with COVID-19 symptoms or with those who had laboratory confirmation of COVID-19 were similarly distributed in the vaccinated and unvaccinated persons without significance differences ($p > 0.05$).

At the second cross-sectional testing, seven days from the first, 24% of the respondents were lost to follow-up, thus, a total of 354 participants were included, of which 150 (42.4%) were vaccinated and 204 (57.6%) were not vaccinated. There was no statistically significant difference in the application of the proposed preventive measures and activities during and after the EXIT festival, with respect to the vaccination status of the participants, as reported in the Table 2.

The majority of participants (68.6%) did not wear a face mask at the festival, but 87.6% reported having worn a mask after the festival and until the moment of testing. Most of the participants avoided staying indoors with a large number of people (78.8%) and applied other pre-

ventive measures, like physical distancing, hand washing and disinfection (92.1%). Only one unvaccinated respondent reported having been in contact with a person with a confirmed COVID-19. That participant also reported the presence of symptoms that may indicate COVID-19 that appeared in the first days after visiting the EXIT festival (fever, sore throat, fatigue, runny nose), but his RDT-Ag was negative at the second cross-sectional testing, performed seven days after visiting the festival.

Table 1 Socio-demographic and epidemiological characteristics of participants tested at the first day of arrival at the EXIT festival, Novi Sad, Serbia

Variables	Total (n=466), n (%)	Vaccination status		p-value*
		Vaccinated (n=170), n (%)	Unvaccinated (n=296), n (%)	
Sex				
Male	250 (53.7)	90 (52.9)	160 (54.1)	
Female	214 (45.9)	79 (46.5)	135 (45.6)	0.905
Missing	2 (0.4)	1 (0.6)	1 (0.3)	
Age (years), mean (SD)	28.34±8.87	31.70±10.54	26.41±7.08	0.001
Age group				
15-20	91 (19.5)	23 (13.6)	68 (23.0)	
21-30	231 (49.6)	75 (44.1)	156 (52.7)	
31-40	89 (19.1)	31 (18.2)	58 (19.6)	<0.001
41-50	44 (9.4)	31 (18.2)	13 (4.4)	
51-60	11(2.4)	10 (5.9)	1 (0.3)	
Marital status				
Single	234 (50.2)	73 (42.9)	161 (54.4)	
In a relationship / extramarital	155 (33.3)	51 (30.0)	104 (35.1)	<0.001
Married	75 (16.1)	44 (25.9)	31 (10.5)	
Missing	2 (0.4)	2 (1.2)	0 (0)	
Level of education				
Primary / secondary school	207 (44.4)	80 (47.1)	127 (42.9)	
Higher education	54 (11.6)	21 (12.3)	33 (11.2)	0.533
University degree	205 (44.0)	69 (40.6)	136 (45.9)	
Having any chronic disease				
Yes	18 (3.9)	12 (7.1)	6 (2.0)	0.007
No	448(96.1)	158 (92.9)	290 (98.0)	
Traveling abroad				
Yes	25 (5.4)	16 (9.4)	9 (3.0)	0.004
No	441 (94.6)	154 (90.6)	287 (97.0)	
Contact with a person/s with COVID-19 symptoms				
Yes	3 (0.7)	2 (1.2)	1 (0.3)	
No	462 (99.1)	168 (98.8)	294 (99.4)	0.415
Missing	1 (0.2)	0 (0)	1 (0.3)	
Contact with the confirmed COVID-19 case/s				
Yes	2 (0.4)	2 (1.2)	0 (0.0)	
No	463 (99.4)	168 (98.8)	295 (99.7)	0.131
Missing	1 (0.2)	0 (0)	1 (0.3)	
Pre-existing COVID-19 infection				
Yes	100 (21.5)	46 (27.1)	54 (18.2)	0.026
No	366 (78.5)	124 (72.9)	242 (81.8)	
Wearing a mask adequately				
Yes	438 (94.0)	165 (97.1)	273 (92.2)	
No	27 (5.8)	4 (2.3)	23 (7.8)	0.024
Missing	1 (0.2)	1 (0.6)	0 (0)	
Adherence to other preventive measures#				
Yes	455 (97.6)	167 (98.2)	288 (97.3)	
No	10 (2.2)	2 (1.2)	8 (2.7)	0.232
Missing	1 (0.2)	1 (0.6)	0 (0)	

*Significant results ($p<0.05$) are presented in bold. #Other preventive measures included: physical distancing, hand washing and disinfection.

Table 2 Adherence to the preventive measures in participants tested seven days after the EXIT festival visit, according to their vaccination status, Novi Sad, Serbia

Variables	Total (n=354) n (%)	Vaccination status		p-value
		Vaccinated (n=150), n (%)	Unvaccinated (n=204), n (%)	
Wearing a mask at the festival				
Yes	110 (31.1)	52 (34.7)	58 (28.4)	
No	243 (68.6)	97 (64.7)	146 (71.6)	0.219
Missing	1 (0.3)	1 (0.6)	0 (0)	
Wearing a mask after the festival and until the moment of testing				
Yes	310 (87.6)	133 (88.7)	177 (86.8)	
No	43 (12.1)	16 (10.7)	27 (13.2)	0.394
Missing	1 (0.3)	1 (0.6)	0 (0)	
Staying indoors with a large number of people				
Yes	74 (20.9)	27 (18.0)	47 (23.0)	
No	279 (78.8)	122 (81.3)	157 (77.0)	0.270
Missing	1 (0.3)	1 (0.7)	0 (0)	
Use of other preventive measures[#]				
Yes	326 (92.1)	141(94.0)	185 (90.7)	
No	26 (7.3)	8 (5.3)	18 (8.8)	0.453
Missing	2 (0.6)	1 (0.7)	1 (0.5)	
Contact with the confirmed COVID-19 case/s				
Yes	1 (0.3)	0 (0.0)	1 (0.5)	0.576
No	353 (99.7)	150 (100.0)	203 (99.5)	

[#]Use of other preventive measures included: physical distancing, hand washing and disinfection

Table 3 Presence of the COVID-19-like symptoms from the day after the EXIT festival, by vaccination status of the participants, Novi Sad, Serbia

Vaccination status against COVID-19	Total (n=354), n (%)	COVID-19-like symptoms from the day after the festival		p-value
		No (n=334), n (%)	Yes (n=20), n (%)	
Vaccinated	150 (42.4)	142 (42.5)	8 (40.0)	0.825
Unvaccinated	204 (57.6)	192 (57.5)	12 (60.0)	

Table 4 Adherence to proposed preventive measures in participants tested seven days after the EXIT festival visit, according to the presence of COVID-19-like symptoms, Novi Sad, Serbia

	Total (n=354), n (%)	Without COVID-19 symptoms (n=334), n (%)	With COVID-19 symptoms (n=20), n (%)	p-value
Wearing a mask at the festival				
Yes	110 (31.1)	106 (31.7)	4 (20.0)	
No	243 (68.6)	227 (68.0)	16 (80.0)	0.523
Missing	1 (0.3)	1 (0.3)	0 (0)	
Wearing a mask after the festival and until the moment of testing				
Yes	310 (87.6)	292 (87.4)	18 (90.0)	
No	43 (12.1)	41 (12.3)	2 (10.0)	0.925
Missing	1 (0.3)	1 (0.3)	0 (0)	
Staying indoors with a large number of people				
Yes	74 (20.9)	73 (21.9)	1 (5.0)	
No	279 (78.8)	260 (77.8)	19 (95.0)	0.189
Missing	1 (0.3)	1 (0.3)	0 (0)	
Using other preventive measures[#]				
Yes	326 (92.1)	308 (92.2)	18 (90.0)	
No	26 (7.3)	24 (7.2)	2 (10.0)	0.847
Missing	2 (0.6)	2 (0.6)	0 (0)	
Contact with the confirmed COVID-19 case/s				
Yes	1 (0.3)	0 (0.0)	1 (5.0)	0.057
No	353 (99.7)	334 (100)	19 (95.0)	

[#]Use of other preventive measures included: physical distancing, hand washing and disinfection

Twenty participants (5.6%), all of which were tested negative at the first RDT-Ag test, reported that they had some of the symptoms that may indicate COVID-19 infection in the first seven days after visiting the festival (Supplementary Table 1). Nasal congestion and runny nose, along with coughing and chest pain were the most common symptoms reported by these participants. Fever, malaise, diarrhoea, nausea, and headache were reported less frequently. In general, subjects reported the presence of multiple symptoms simultaneously. No significant difference in vaccination status was observed between those who reported having some of the symptoms that may indicate COVID-19 infection (40% were vaccinated and 60% were unvaccinated) and those who did not (42.5% were vaccinated and 57.5% were unvaccinated) ($p = 0.825$) (Table 3). Finally, among these participants, tested by the RDT-Ag test after seven days after visiting the EXIT festival, no COVID-19 infection was confirmed.

Adherence to several preventive measures in participants with and without COVID-19-like symptoms is reported in the Table 4.

No statistical difference ($p>0.05$) was found in behaviors like wearing a mask at the festival, wearing a mask after the festival and until the moment of testing, staying indoors with a large number of people, using other preventive measures, and contact with the confirmed COVID-19 case/s, between the two observed groups. Finally, there was no statistically significant difference ($p = 0.173$) in the mean hours spent during the festival between visitors with (22.55 ± 15.35 hours) and those without COVID-19-like symptoms (18.06 ± 14.24 hours) (data are not shown).

Discussion

The results of our analysis indicate that in the group of respondents negative on the first antigen test performed on the first day of their visit to the EXIT festival, during which they applied strict measures to prevent the spread of SARS-CoV-2 virus, there was no confirmed COVID-19, by the RDT-Ag testing, in the period of seven days after the visit to the festival. Specifically, at the second screening using RDT-Ag test, which was performed on the seventh day after the first, the test results remained negative in all subjects regardless of their vaccination status. About 5% of respondents reported symptoms that may indicate COVID-19 infection within seven days of visiting the festival, but the result of an RDT-Ag in these subjects remained negative.

Our results coincide with the results of several studies published worldwide [9, 19–21]. Namely, it was noticed that mass gathering outdoor events, regardless of the location, if organized in a way that allows strict control of the SARS-CoV-2 virus transmission and application of all prescribed preventive measures, carry a low risk of the SARS-CoV-2 transmission [9, 19–21]. A recent systematic review, which included 11 studies examining the risks of COVID-19 in visitors to various mass events, indicated that strict application of preventive measures and their control can reduce, but not completely eliminate the risk for SARS-CoV-2 transmission among visitors [9]. Using mathematical models and data from studies conducted so far, it is estimated that an hour of stay at the theater performances, business conferences, football matches or an outdoor music events leads to 0.6 to 1.7 COVID-19 infections per 100,000 visitors, while an hour time spent at dance events and indoor concerts and festivals where visitors dance outdoors leads to 1.8 to 4.3 COVID-19 infections per 100,000 visitors [9]. Several studies emphasized the importance of a multilevel approach to the control of SARS-CoV-2 transmission, which involves the application of several preventive measures simultaneously. Thus, it becomes essentially difficult to determine the effectiveness of any measure individually [9, 24–30]. In our study, we noticed that there was no difference in the occurrence of the disease, more precisely, there was no confirmed COVID-19 infection in the participants of our study, regardless of their vaccination status.

At most mass events in the world, preventive measures that provide means for hand disinfection, wearing face masks, providing adequate ventilation, health examination and contact tracing are implemented [24–31]. In certain circumstances, the quarantine measure before visiting the event was applied to the visitors of mass events [25, 27, 31]. With increasing coverage of immunization against COVID-19 and the availability of different vaccines, the approach to organizing mass events has changed [9]. From June 2021, many countries around the world began to "open up" and allow the organization of mass events, requiring visitors to have a certificate of vaccination, or a certificate of a previous COVID-19 infection not older than six months, or a negative RDT-Ag or PCR test not older than 48 hours [9, 19–21].

It has been previously known that the use of RDT-Ag helps in the control of the COVID-19 in schools, in professional sports competitions, in health and social institutions, and mass gatherings around the world [11, 21, 32, 33]. Although data on the high effectiveness of the RDT-Ag strategy are increasingly available, caution should be placed when interpreting test results, as the sensitivity of RDT-Ag, especially in asymptomatic individuals widely ranged from 41.2% to 93.3%, depending on the type of RDT-Ag [11, 34–36]. Also, the sensitivity of the rapid antigen test is lower if the prevalence of COVID-19 in the population is lower, so repeated testing of the population of interest is recommended in such situations in order to increase the sensitivity of the applied RDT-Ag for the virus detection [11,34–36]. On the other hand, RDT-Ag testing is a very useful method, because the results are quickly available (approximately after 10-15 minutes), due to their high specificity (positive persons can be immediately classified as positive) can be used on the swab sampling site (“point-of-care”, “near patient”), while the use of PCR testing such as the gold standard, is much more expensive, requires more time, special equipment and machines, and specially trained staff [11, 18, 34–36].

Results from several studies indicate that the application of a one-day screening RDT-Ag the day before a concert or other mass event, the use of face masks and adequate and forced room ventilation can prevent high rates of SARS-CoV-2 infection in the organization of a mass event indoors, and in situations where maintaining the physical distance among visitors is not possible and when vaccination coverage in the population is low, i.e. less than 10% [19, 20, 36]. However, the results of various studies have shown that mass gatherings with preventive measures have a limited impact on SARS-CoV-2 virus transmission and on the increase of the COVID-19 incidence in the population, if the current incidence in the community is low [9, 36]. Otherwise, the impact of mass gathering on SARS-CoV-2 transmission and an increase in COVID-19 morbidity in the population would be significant and could contribute to an increase in morbidity of up to about 23% [9, 36]. A major problem around the world is the spread of the new variant of the virus, like delta and omicron variants, which were found to be more contagious than previous variants of SARS-CoV-2 virus and that also could break through the immune barrier acquired after COVID-19 infection or vaccination [16,37]. Thus, it is very important to consider this when organizing mass events, given that during July 2021, when organizing several major public events in Massachusetts, a slight increase in the incidence of COVID-19 was observed among the residents, even among those vaccinated, and in 90% of patients from the sample, the delta variant of SARS-CoV-2 was isolated [5].

Our study has some limitations. Firstly, we had no control group of respondents who did not attend the EXIT festival and therefore we could not compare the differences in disease rates between the exposed to the event and those that were not exposed. Secondly, even though the EXIT festival is an international festival with visitors from all over the world, we included only visitors that are residents of Serbia, since it was necessary to conduct a repeated RDT-Ag testing seven days after the festival ended, which would not be possible to conduct for the international visitors since the majority of them travelled back to their homeland countries as soon as the festival finished. Thirdly, during the time of the festival, the number of COVID-19 cases in the population of the city of Novi Sad as well as the Republic of Serbia was low, and thus the circulation of the virus in the population was low, which could indirectly affect the lower risk of spreading the virus among EXIT festival visitors and consequently the absence of the COVID-19 [38]. Also, the low prevalence of COVID-19 in the population at the time of the study could potentially affect the lower sensitivity of the RDT-Ag. Finally, a slightly smaller number of subjects responded to the follow-up call for the second RDT-Ag testing compared to the calculated sample size However, we argue that this reduction in sample size did not significantly affect our findings.

Despite these limitations, the results of our study and their comparability with the previous findings from the literature are important since they imply the possibility of a good control of the SARS-CoV-2 transmission and COVID-19 at the mass events during the COVID-19 pandemic. Also, our findings indicate that the organization of mass events is accompanied by a low risk of disease if the recommended preventive measures are applied and strictly controlled. In line with this, there was no increased number of COVID-19 cases in Novi Sad, in community, after the EXIT festival finished. The results of our study should be interpreted in the light of the intensity of SARS-CoV-2 virus transmission in the population, the emergence of new virus variants and their characteristics. Finally, current results lead to the conclusion that with good organization and following the preventive measures during a visit to a mass event, the risk of COVID-19 associated with a mass event is not significantly higher than that estimated in the population, at the time.

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Original Scientific Article

USE OF BISPECTRAL INDEX (BIS) FOR MONITORING OF SEDATION AND TOTAL INTRAVENOUS ANESTHESIA (TIVA) IN PEDIATRIC PATIENTS UNDERGOING COLONOSCOPY

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Abstract. *The objectives of this study were to determine whether there was a correlation between bispectral index (BIS) and Ramsey Sedation Scale (RSS) in regard to the type of sedation and total intravenous anesthesia (TIVA) during colonoscopy procedures in children, and to assess the utility of ketamine and propofol combination (ketofol) for this kind of procedures at children's age. In our prospective study, 40 ASA I-II patients, 3 to 17 years of age, were randomly divided into two groups of 20 patients each. After premedication with atropine and midazolam, sedation was induced with propofol and fentanyl in Group PF, whereas in Group PK propofol and ketamine were used for induction. Both groups were further divided into two subgroups depending on whether anesthesia was maintained with intermittent doses or continuous infusion of propofol. Ketamine and/or fentanyl were administered as bolus doses. Heart rate (HR), peripheral oxygen saturation (SpO2), RSS and BIS values of all patients were recorded every 5 minutes throughout the colonoscopy procedures. The strongest degree of correlation between RSS and BIS existed when sedation or TIVA was maintained by the boluses of propofol and fentanyl. The use of ketamine significantly reduced the doses of propofol and fentanyl. BIS can be monitored in all pediatric patients in whom sedation and TIVA are administered during colonoscopy, but the effect of different anesthetics on the EEG signal should be considered in order to adequately assess the depth of sedation and anesthesia*.

Key words: *awareness, monitoring, child, anesthetics, endoscopy*

Introduction

Awareness during anesthesia is a serious complication with potentially long-term psychological consequences. In practice, about 95% of the cases of consciousness are blamed on human error, the wrong anesthetic technique, or the malfunction of the anesthesia machine [1, 2].

Monitoring of the bispectral index (BIS) enables the reduction of the incidence of awareness during sedation or general anesthesia. It is considered a valuable monitor of sedation levels and loss of consciousness for a wide range of anesthetics, such as propofol, midazolam and sevoflurane. BIS monitoring has also become a helpful tool to titrate hypnotic agents and reduce drug consumption, therefore allowing faster recovery while avoiding side effects such as hemodynamic instability [3].

The efficacy of BIS monitoring during sedation and total intravenous anesthesia (TIVA) for colonoscopy in children is debated for two reasons. In the first place, the influence of different anesthetics applied during sedation and anesthesia should be considered, eg ketamine can lead to a transient increase in BIS values due to activation of electroencephalogram (EEG), etomidate-induced myoclonus also transitory increases the BIS value [4]. Another important question that arises is whether BIS can be equally applied to children who are subjected to colonoscopy in the same way as it is used in adults? Children cannot be expected to participate in volunteer studies involving sedation and general anesthesia. Estimates that depend on the response to the verbal command or memory function are unreliable in this population. And in the waking state, from infant to adulthood, EEG amplitude decreases and the frequency of brain activity increases. In addition, EEG during anesthesia, especially in infants, differs from adults because the maturation of the brain tissue and the formation of synapses occurs in the first months of life [5-7].

The objectives of this study were to 1) determine whether there was a correlation between BIS and Ramsey Sedation Scale (RSS) in regard to the type of sedation and total intravenous anesthesia (TIVA); 2) assess the utility of ketamine and propofol combination (ketofol); 3) compare doses of drugs when used in different combinations for colonoscopy procedures in children.

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Patients and Methods

In our prospective study, after obtaining the Ethics Committee approval (No 5343/15, March 1, 2016, according to the Article 12 Rules of Procedure of the Ethics Committee Clinical Centre Nis) and written informed consent from the parents, 40 ASA I-II patients, 3 to 17 years of age, were randomly divided into two groups of 20 patients each. After premedication with atropine (Atropina solfato S.A.L.F.®; Laboratorio Farmacologico, Bergamo, Italy) and midazolam (Dormicum®; Roche, Basel, Switzerland), sedation was inducted with 1 mg/kg propofol (Propofol 1% Fresenius®; Fresenius Kabi, Graz, Austria) + 1 mcg/kg fentanyl (Fentanyl Panpharma®, Rotexmedica, Trittau, Germany) in Group PF, and 1 mg/kg propofol + 1 mg/kg ketamine (<20 kg BW) or 0.5 mg/kg ketamine (>20 kg BW), (Ketamine hydrochloride®; Rotexmedica, Trittau, Germany), in Group PK. Both groups were divided into two subgroups. In PF1 Group, deep sedation was maintained with boluses of propofol and fentanyl, whereas in Group PF2 sedation was maintained with continuous infusion of propofol (3 mg/kg) with intermittent boluses of fentanyl and propofol. In PK1 Group deep sedation was maintained with intermittent boluses of propofol, ketamine, and fentanyl, while in Group PK2 sedation was maintained using continuous infusion of propofol (3 mg/kg) with intermittent boluses of ketamine and fentanyl.

Heart rate (HR), peripheral oxygen saturation (SpO2), RSS (Table 1) and BIS values (BIS VISTA™ monitoring system, Aspect Medical Systems, Inc., the Netherlands) of all patients were recorded throughout the colonoscopy procedures. The observer who assessed sedation using RSS did not communicate his assessment to those who administered the drugs and recorded BIS values.

Table 1 Ramsay Sedation Scale

Definition	Score
Patient is anxious and agitated or restless, or both	1
Patient is cooperative, oriented and calm	2
Patient responds to commands only	3
Patient exhibits brisk response to light glabellar tap or loud auditory stimulus	4
Patient exhibits a sluggish response to light glabellar tap or loud auditory stimulus	5
Patient exhibits no response	6

Colonoscopy duration included the overall time of the endoscopy procedure. The recovery time was related to the fall of the RSS score to 2. The discharge time was referring to the transfer of a patient from the post anesthesia care unit (PACU) to the pediatric gastroenterology unit. Pediatric gastroenterologist's satisfaction was scored on a visual analogue scale (VAS) from 1 to 10.

Statistical Analysis

Statistical analysis of data was performed with SPSS 15.0 statistic software package (SPSS, Chicago, IL, USA). Continuous variables were presented as arithmetic mean ($\bar{X}$), standard deviation (SD) and median (Me). The qualitative characteristics of the examined variables were presented as frequency (n) and percentage value (%). The regularity of the distribution of the continuous variables, depending on the sample size, was examined by the Shapiro-Wilk test. The Student's t-test was used for normally distributed parameters, whereas for non-normally distributed variables, the Mann–Whitney U-test was used to compare the two groups. A value of $p < 0.05$ was considered significant. As a measure of the linear relationship between two continuous variables Pearson correlation coefficient was used for normally distributed variables whereas nonparametric Spearman's rank correlation coefficient provided a measure of a monotonic relationship between variables that were not normally distributed.

Results

There were no significant differences between the groups in age, weight, gender, American Society of Anesthesiologists (ASA) Score and the duration of colonoscopy (Table 2).

Figure 1 shows that in most of the observed periods the highest SpO_2 values were in the PK1 Group (intermittent boluses of propofol, ketamine, and fentanyl), where these values were statistically significantly higher in relation to PF2 (continuous infusion of propofol with intermittent boluses of fentanyl and propofol) in the 1st and 25th minute ($p < 0.05$), as well as in relation to PF2 and PK2 (continuous infusion of propofol with intermittent boluses of ketamine and fentanyl) in the 40th and 45th minute ($p < 0.01$). In the 40th minute, the value in the PF1 Group (boluses of propofol and fentanyl) was higher than in the PF2 and PK2 Group ($p < 0.01$).

Table 2 Demographic characteristics of the patients and duration of colonoscopy

		PF1	PF2	PK1	PK2
Age (years)		12.20 ± 4.73 (14.00)	12.20 ± 4.34 (13.00)	11.50 ± 4.77 (12.00)	12.60 ± 5.66 (15.00)
Weight (kg)		52.70 ± 21.95 (60.50)	43.10 ± 9.50 (43.00)	41.60 ± 12.02 (41.50)	41.80 ± 15.26 (49.00)
Colonoscopy duration (min)		53.00 ± 10.06 (57.50)	56.50 ± 5.30 (60.00)	52.00 ± 10.33 (55.00)	55.30 ± 7.59 (60.00)
Gender	M	5 (50.00%)	6 (60.00%)	5 (50.00%)	6 (60.00%)
	F	5 (50.00%)	4 (40.00%)	5 (50.00%)	4 (40.00%)
ASA	1	4 (40.00%)	4 (40.00%)	4 (40.00%)	5 (50.00%)
	2	6 (60.00%)	6 (60.00%)	6 (60.00%)	5 (50.00%)

Notes: Continues variables are given as means ± SD (medians) and categorical variables as absolute number and in percentages (%)
Abbreviations: ASA, American Society of Anesthesiologists Score

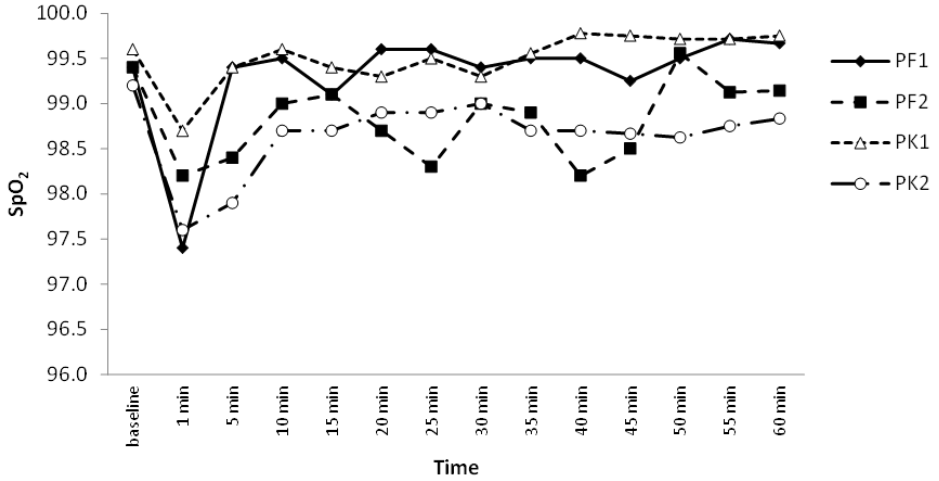

Fig. 1 SpO_2 values recorded during colonoscopy procedure.

Abbreviations: SpO_2, peripheral capillary oxygen saturation; PF1, boluses of propofol and fentanyl; PF2, continuous infusion of propofol (3 mg/kg) with intermittent boluses of fentanyl and propofol; PK1, boluses of propofol, ketamine, and fentanyl; PK2, continuous infusion of propofol (3 mg/kg) with intermittent boluses of ketamine and fentanyl.

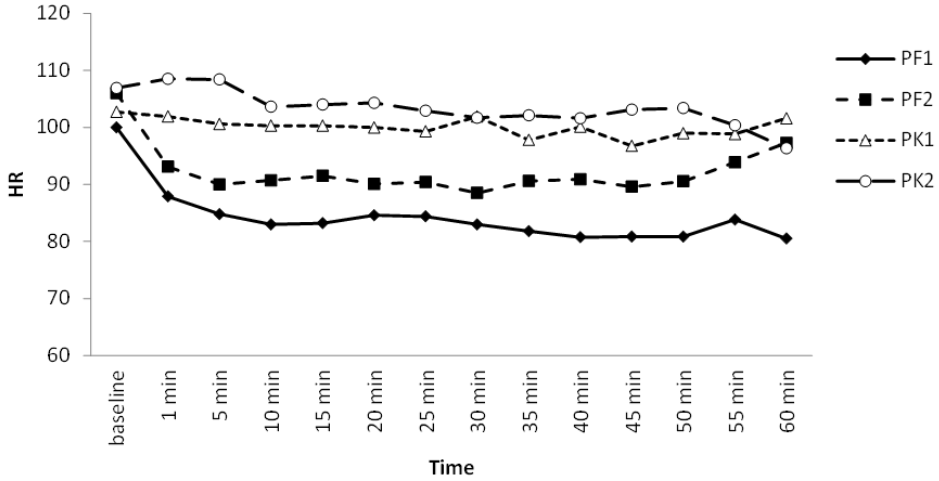

Fig. 2 HR values recorded during colonoscopy procedure.

Abbreviations: HR, heart rate; PF1, boluses of propofol and fentanyl; PF2, continuous infusion of propofol (3 mg/kg) with intermittent boluses of fentanyl and propofol; PK1, boluses of propofol, ketamine, and fentanyl; PK2, continuous infusion of propofol (3 mg/kg) with intermittent boluses of ketamine and fentanyl.

By analyzing the data in Figure 2, it is evident that, except in the 30th and 60th minute, the heart rate (HR) values were the highest in the PK2 Group, while the values in both PF Groups were lower, which was most pronounced in the PF1 Group. Compared to the PF1, the HR values were statistically higher in the PK1 Group at 5, 10. 15, 20 and 30 minutes, the same applied to the PK2 Group in the 1st and 5th minute ($p < 0.05$).

Figure 3 shows the RSS values for the investigated groups. The values of this parameter were relatively close among the groups, and only in 5th minute significantly higher RSS was noticed in the PF2 Group compared to the PK2 Group ($p < 0.05$).

The data presented in Figure 4 indicate that BIS values in the PK1 Group were statistically significantly higher in relation to the PF2 Group in 5, 10 and 15 minutes ($p < 0.05$), as well as in the 25, 30 and 40 minutes ($p < 0.01$), the same pattern repeated in relation to the PF1 Group at 15, 20, 25, 30, 35 and 40 minutes ($p < 0.05$). The BIS values were higher in the PK2 Group compared to the PF2 group in 5 ($p < 0.01$), 10 and 15 minutes ($p < 0.05$), and in relation to the PF1 Group at 20 and 30 minutes ($p < 0.05$). Generally, the values were lower in the PF1 and PF2 Groups. In 1st minute, BIS in PF1 was statistically significantly lower compared to all three other groups ($p < 0.05$).

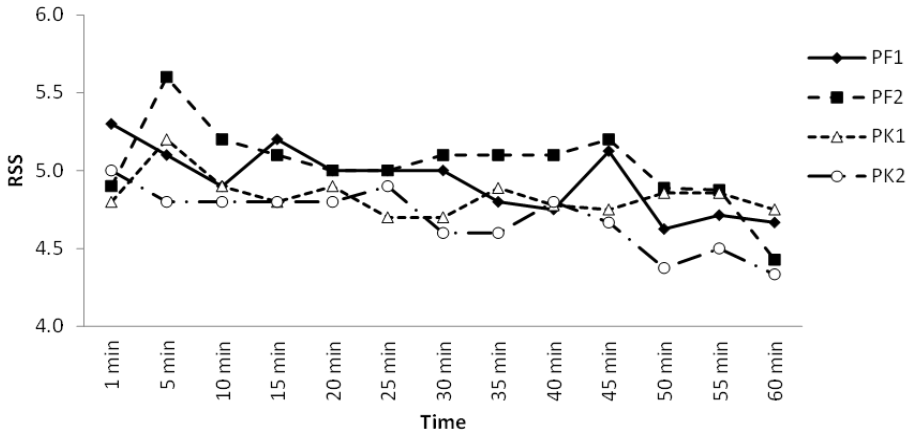

Fig. 3 RSS monitored during colonoscopy procedure.

*Abbreviations***:** RSS, Ramsay Sedation Scale; PF1, boluses of propofol and fentanyl; PF2, continuous infusion of propofol (3 mg/kg) with intermittent boluses of fentanyl and propofol; PK1, boluses of propofol, ketamine, and fentanyl; PK2, continuous infusion of propofol (3 mg/kg) with intermittent boluses of ketamine and fentanyl.

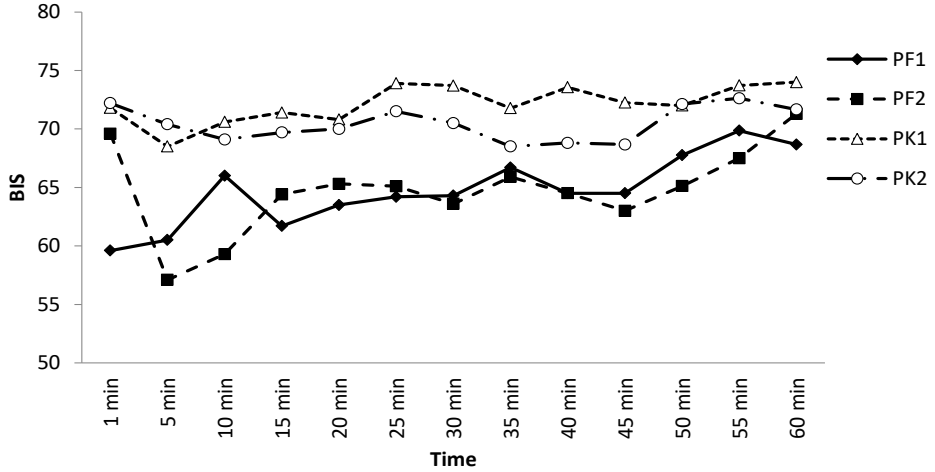

Fig. 4 BIS values measured during colonoscopy procedure.

*Abbreviations***:** BIS, bispectral index; PF1, boluses of propofol and fentanyl; PF2, continuous infusion of propofol (3 mg/kg) with intermittent boluses of fentanyl and propofol; PK1, boluses of propofol, ketamine, and fentanyl; PK2, continuous infusion of propofol (3 mg/kg) with intermittent boluses of ketamine and fentanyl.

Table 3 Correlations between RSS and BIS values

	PF1	PF2	PK1	PK2
1 min	-0.87 **	-0.81 **	-0.70 *	-0.46
5 min	-0.76 *	-0.71 *	-0.86 **	-0.57
10 min	-0.76 *	-0.70 *	-0.82 **	-0.44
15 min	-0.92 ***	-0.28	-0.52	-0.57
20 min	-0.96 ***	-0.55	- 0.18	-0.51
25 min	-0.77 **	-0.86 **	-0.58	-0.64 *
30 min	-0.87 **	-0.81 **	-0.70 *	-0.51
35 min	-0.85 **	-0.81 **	-0.48	-0.42
40 min	-0.51	-0.66 *	-0.74 *	-0.81 **
45 min	-0.79 *	-0.83 **	-0.76 *	-0.91 ***
50 min	-0.69	-0.40	-0.62	-0.77 *
55 min	-0.88 **	-0.50	-0.62	-0.87 **
60 min	-0.93 **	-0.67	-0.77	-0.83 *

Notes: * – p<0.05, ** – p<0.01, *** – p<0.001

Abbreviations: PF1, boluses of propofol and fentanyl; PF2, continuous infusion of propofol (3 mg/kg) with intermittent boluses of fentanyl and propofol; PK1, boluses of propofol, ketamine, and fentanyl; PK2, continuous infusion of propofol (3 mg/kg) with intermittent boluses of ketamine and fentanyl.

Correlations between RSS and BIS values for all examined periods and groups are presented in Table 3. As expected, due to the fact that the higher value of RSS means that the patient sleeps deeper, and the lower BIS means that anesthesia is deeper, in all periods of observation correlations were negative. It is also evident that patients within the PF1 Group had the largest number of such correlations.

The mean total propofol dose in mg/kg (Table 4) was significantly lower ($p < 0.001$) in PK1 Group (1.86) than in PF1, PF2 and PK2 Groups (4.06, 4.99 and 3.95, respectively). The mean fentanyl dose (mcg/kg) was the highest ($p < 0.01$) in PF2 Group (2.06) in comparison to PF1, PK1 and PK2 Groups (1.60, 1.46 and 1.39, respectively). No significant difference was noted in mean ketamine dosage (mg/kg) between PK1 and PK2 Groups (1.24 and 1.33, respectively). Recovery time (min) was significantly shorter in PF1 Group (14.20) and in PF2 Group (14.40) in comparison with PK1 and PK2 Groups (17.30 and 16.50, respectively). Discharge

Table 4 Distribution of propofol, fentanyl and ketamine doses, rate of complications, recovery and discharge times and colonoscopist satisfaction score

	PF1	PF2	PK1	PK2
Total propofol dose (mg)	204.00 ± 81.13 (205.00)^{c***}	212.90 ± 53.32 (220.00)^{c***}	76.50 ± 24.04 (72.50)	160.60 ± 52.56 (184.00)^{c**}
Propofol dose (mg/kg)	4.06±1.03 (3.61)^{c***}	4.99±0.97 (5.13) ^{c***d**}	1.86±0.28 (1.83)	3.95±0.50 (3.97) ^{c***}
Total fentanyl dose (mcg)	85.00 ± 42.82 (87.50)^{d*}	88.50±34.32 (75.00)^{c*d**}	58.50±16.67 (57.50)	47.20±20.29 (42.50)
Fentanyl dose (mcg/kg)	1.60 ± 0.58 (1.43)	2.06 ± 0.66 (1.86)^{acd**}	1.46 ± 0.44 (1.35)	1.39 ± 0.64 (1.20)
Total ketamine dose (mg)			49.00±8.76 (50.00)	47.20±20.29 (42.50)
Ketamine dose (mg/kg)			1.24±0.34 (1.22)	1.33±0.80 (1.33)
Complications	2 (20.00%)	2 (20.00%)	0 (0.00%)	2 (20.00%)
Recovery time (min)	14.20±2.30 (14.00)	14.40±2.41 (15.00)	17.30±1.64 (17.00)^{ab**}	16.50±2.59 (15.00)^{a*}
Discharge time (min)	34.30±3.92 (33.50)	36.80±7.45 (34.50)	49.10±5.82 (47.00)^{a***b**}	50.00±7.36 (49.00)^{a***b**}
Gastroenterologist satisfaction	9.90±0.32 (10.00)	9.80±0.42 (10.00)	9.90±0.32 (10.00)	9.90±0.32 (10.00)

Notes: Continues variables are given as means ± SD (medians) and categorical variables as absolute number and in percentages (%); a – vs PF1, b – vs PF2, c – vs PK1, d – vs PK2; * – p<0.05, ** – p<0.01, *** – p<0.001.
Abbreviations: PF1, boluses of propofol and fentanyl; PF2, continuous infusion of propofol (3 mg/kg) with intermittent boluses of fentanyl and propofol; PK1, boluses of propofol, ketamine, and fentanyl; PK2, continuous infusion of propofol (3 mg/kg) with intermittent boluses of ketamine and fentanyl.

time (min) was significantly longer in PK1 and PK2 Groups (49.10 and 50.00) in comparison to PF1 and PF2 Groups (34.30 and 36.80). Only in the PK1 Group we did not note transitory respiratory depression. No other adverse events were noted in any of the investigated groups.

Discussion

A colonoscopy is uncomfortable for the patient because of its long duration and many factors affecting abdominal pain.[5] In children, colonoscopy presents an even greater challenge. Achieving and maintaining an adequate level of sedation is difficult, therefore, it is important to set an objective indicator for monitoring the patient sedation level during colonoscopy [8-10].

The bispectral index algorithm was developed by recording and retrospectively analyzing EEG data of healthy adults, who suffered a repeated transition between a conscious and unconscious state using several different anesthetic regimens. BIS monitor generates a number on an uninterrupted scale from 0 to 100, where 100 represents normal cortical electrical activity, 90-60 sedation, 60-40 general anesthesia, 40-20 deep anesthesia and 0 represents the absence of any activity. The precondition for the correct interpretation of BIS changes is the knowledge of the specific effects of anesthetic agents on the EEG [4,11]. Meta-analyses conducted by Park et al [12] showed that the total propofol consumption was significantly lower in the BIS group than in the non-BIS group, although mean propofol consumption was not significantly different. In the pediatric population, the ability of the BIS to accurately follow variations in anesthetic agent concentration and evaluate depth of anesthesia remains controversial. Tirel et al [13] found the large variation of BIS values at 2 mcg/ml target-controlled plasma propofol concentration and explained that it could be mainly due to the influence of age. The effect of fentanyl on the BIS value was described as minimal, although its administration is associated with clinical evidence of increased sedation [14]. In the present study, fentanyl was administered along with other drugs such as midazolam, propofol or ketamine and so we cannot comment on the effect of fentanyl alone on the BIS values. Ketamine, in the doses of 0.25 to 0.5 mg/kg, can block the response capacity of patients, but it does not reduce the BIS [15]. Vereeke et al [16] found that BIS values increased significantly between 3 and 8 minutes after administration of ketamine bolus followed by a subsequent decrease for the rest of the study period. Faraoni et al [17] concluded that during stable propofol-remifentanil anesthesia low doses of ketamine (0.2 mg/kg) had no effect on BIS.

Without any doubt, the depth of sedation should primarily be monitored on the basis of clinical criteria [18] so studies were conducted to determine the correlation between different sedation scores and BIS values. Sadhasivam et al [14] described a significant correlation between BIS and Observer's Assessment of Alertness/Sedation (OAA/S) as well as between BIS and University of Michigan Sedation Scale (UMSS). The

authors excluded children requiring sedation with ketamine. In our study, although BIS values were higher in patients who received ketamine, we found correlation between BIS and RSS in all investigated groups. This could be in line with the study of Vereecke et al [16] who concluded that when used during sedation in combination with propofol, ketamine increased hypnosis without affecting BIS levels. Our results showed that the strongest degree of correlation between RSS and BIS existed when TIVA was maintained by the boluses of propofol and fentanyl.

We would like to emphasize the importance of propofol and ketamine combination ("ketofol") for a comfortable performance of colonoscopy and to give our own contribution regarding its use in pediatric patients. Ketamine stimulates the cardiorespiratory system, as it was also observed in our study because the increased heart rate was recorded in patients in whom ketamine was administered. It also increases cardiac output, arterial blood pressure, heart rate and central venous pressures. However, psychomimetic activity, emergence delirium and other adverse events have been shown to be dose related. In contrast, propofol is a sedative, hypnotic and anesthetic agent and it can improve sedation scores but it has a narrow therapeutic range and increases the risks of cardiovascular depression and airway compromise [19,20]. Combining these two agents for colonoscopy may preserve sedation efficacy while minimizing their respective adverse effects. Although popular for short procedural sedation and analgesia in pediatric patients [21] it is surprising that yet neither the optimal combination nor infusion rate of ketofol is known. Coulter et al [22] suggested an optimal ratio of racemic ketamine to propofol of 1 : 5 for 30-min anesthesia and 1 : 6.7 for 90-min anesthesia. Tosun et al [23] investigated propofol-ketamine for sedation during pediatric upper gastrointestinal endoscopy (PK Group received 1 mg/kg ketamine + 1.2 mg/kg propofol with additional propofol (0.5–1 mg/kg) when a patient showed discomfort) and concluded that this combination resulted in stable hemodynamics and deeper sedation but caused more side effects (eg vomiting, dizziness, diplopia). Türk et al. [24] investigated the use of ketofol (prepared at a ratio of 1:2) compared with an opioid-propofol combination in colonoscopic procedures. They reported that ketofol provides better hemodynamic stability and better quality of sedation-analgesia than alfentanil-propofol in elective colonoscopy. In our study, the use of ketamine did not affect the trends of BIS values to the extent that it could lead to a wrong assessment of the clinical depth of sedation or anesthesia, while significantly reducing the doses of propofol and fentanyl. Higher doses of propofol when it was administered as continuous infusion suggested why most gastroenterologists prefer the flexibility of the bolus approach [25]. Only in the group of patients who received appropriate bolus doses of propofol, ketamine and fentanyl (Group PK1) we did not note transitory respiratory depression. No other adverse events were noted in any of the investigated groups. Recovery time was slightly prolonged in patients who received propofol-ketamine combination.

Conclusion

BIS can be monitored in all pediatric patients in whom sedation and TIVA are administered during colonoscopy, but the effect of different anesthetics on the EEG signal should be considered in order to adequately assess the depth of sedation and anesthesia.

The combination of ketamine and propofol for use in procedural sedation has received significant attention during the last few years. Based upon the results of our study, we may conclude that a combination consisting of appropriate doses of propofol, ketamine and fentanyl can be safely used for colonoscopy sedation or TIVA in children.

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Original Scientific Article

OVERCOMING FEAR AVOIDANCE BEHAVIORS AND KINESIOPHOBIA IN PATIENTS WITH CHRONIC LOW BACK PAIN

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Abstract. *Chronic low back pain (CLBP) is a very complex medical, social and economic problem. Long duration and variability of symptoms can be frustrating for patients and lead to various psychological and behavioral changes, which can be expressed as an over-powering fear of movement and lead to avoidance behavior. The aim of this study was to highlight the importance of individually designed exercises (IDE) and cognitive-behavioral treatment (CBT) in the treatment of patients with CLBP who have signs of fear-avoidance behavior and kinesiophobia. One hundred and thirty patients were included in a prospective randomized study. Group 1 (G1; n = 35) had a combined IDE and CBT program. Group 2 (G2; n = 35) had IDE, without CBT. Group 3 (G3; n = 30) had standard group exercises for CLBP. Group 4 (G4; n = 30) was a control, patients did not have IDE or CBT. Waddel's Fear Avoidance Beliefs Questionnaire (FABQ) and Tampa Scale of Kinesiophobia (TSK), were used for monitoring and evaluation. Patients completed them at the beginning, at the end of the therapy, and also after 3 months. After therapy and three months later, a statistically significant reduction in symptoms in G1 and G2 was recorded, with better results in G1: FABQph = 4.77±3.83/5.51±4.02; FABQw = 2.31±3.69/2.94±4.19; TSK = 5.63±4.56/5.69 ± 4.55. (p <0.001). The combination of IDE and CBT is an effective therapy for CLBP patients with fear avoidance behaviour and kinesiophobia*.

Key words: *chronic back pain, exercises, cognitive-behavioral treatment, fear of movement*

Introduction

Rest and protective positions applied in the post-acute period of CLBP are desirable, adaptive responses to pain. However, maintaining this model of behavior for a longer time is unnecessary and can be harmful. The belief that any physical activity can worsen the patients` condition by reactivation of the initial injury often turns into a feeling of fear, which interferes with the rehabilitation process. The patient becomes preoccupied with his/her physical symptoms and turns all of the attention to the feeling of pain, to the extent that he/ she refuses to move and avoids performing any exercises. That kind of behavior leads to the progression of immobility and worsening of pain and disability. It also disrupts the healing process.

Excessive attention to each movement can lead to strict selection of activities of everyday life and avoidance of any movements that the patient believes can provoke or aggravate initially experienced pain, which usually lead to overprotective behavior and harmful inactivity. The abovementioned was recognized and explained back in 2000 with the first fear-avoidance model (Fig.1). Since then, the concept and applicability of this model has changed significantly. The main contribution was in the explanation of why the acute painful lumbar syndrome in some patients turned into chronic, and also in the possibility of early identification of those patients. The modern concept of fear-avoidance model connects individually experienced pain intensity, overwhelming feeling of pain - catastrophizing, exaggerated preoccupation with pain, behavioral disorders such as withdrawal and avoidance, disability, pathological passivity and excessive vulnerability / hypersensitivity. The connection between fear and anxiety on the one hand and chronic pain on the other can be seen through three dimensions: the fear of pain itself, the fear of work-related activities and the fear of certain movements that the patient recognizes as harmful [1].

Fear Avoidance Behaviors

In an attempt to explain how and why some patients develop chronic pain syndrome, Lethem et al, in 1983 [2], introduced the fear-avoidance model. The central point of this initial model was the fear of pain. *Confrontation* and *avoidance* were opposed as the ultimate, extreme, positive and negative responses to that fear. Avoidance as an undesirable reaction to pain that leads to persistence of symptoms and excessively increased feelings of fear that sometimes leads the patient into a phobic state. Avoidance behaviors cause a reduction in physical and social activities, which negatively influence patients` physical and mental condition, promote greater disability and disrupt the overall quality of life. It was noted that some CLBP patients were afraid of pain itself but also of various activities which they believed could cause even

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greater pain than one they were experiencing. Ten years after the first presented fear-avoidance model, Waddel et al. showed in their studies that only the patient's beliefs on harmful effects of physical and work- related activities could cause more damage to the ability to perform activities of daily living and also more frequent absence from work, than the pathoanatomical causes of pain itself, its duration and intensity. The same authors developed the Fear-Avoidance Beliefs Questionnaire (FABQ), which is a powerfull assessesment tool of the impact that fear of movement and work-related activities have on the overall functioning in CLBP patients [3].

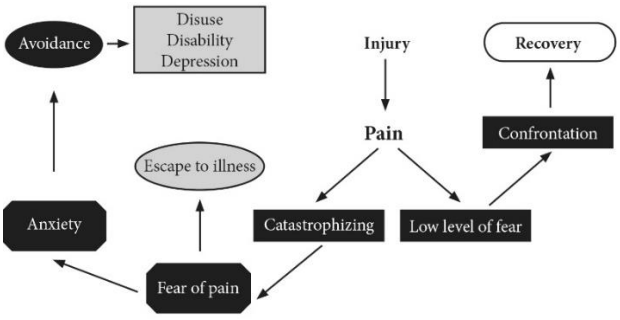

Fig. 1 Fear avoidance model of musculoskeletal pain (modified according to Asmundson et al. – based on the 2000 Vlaeyen / Linton fear-avoidance model) [4, 5]

Kinesiophobia

Special attention should be paid to a specific type of fear – the fear of movement and physical activities that the patient believes may cause or worsen an existing injury and aggravate pain. Kinesiophobia (kinesis - movement) as a term was introdused in 1990. This phenomenon was defined as: "excessive, irrational and incapacitating fear of physical movement and certain, from the patient perspective, risky activities, which is a consequence of the patient's vulnerability and higher exposure to re-injury and accompanying pain." [6]

Fear is a natural, immediate consequence of pain, and avoiding activities and movements that can reactivate the initial event is a normal response to acute pain, but can be a serious obstacle to recovery in the case of chronic pain syndrome. Anxiety and fear that often accompany chronic pain syndromes intensify and multiply the patients` experience of pain. Patients with CLBP who have an increased level of pain-related anxiety, tend to constantly expect even more severe pain and to fear re-exacerbation in advance. That kind of behavior usually leads to negative behavioral changes, most often in terms of reduced physical activity [7]. When patients with CLBP are exposed to a situation that frightens them (which can be climbing stairs, riding a bus, walking, going to the theater, etc.), they will show a kind of avoiding cognitive-behavioral response and excessive concern for their health, in a natural attempt to avoid a potentially risky situation and prevent pain aggravation and exacerbation of accompanied symptoms [8, 9].

Waddell et al. [3] suggested that avoiding the activities of everyday life and professional obligations was directly related to the degree of professional incapacity and job loss, regardless of the severity of the condition itself and the true intensity of the accompanying pain. The sentence from the study conducted by Crombez et al. [7]: “fear of pain and what we do about it is more disabling than the pain itself”, best explains this phenomenon. Subsequent studies have shown that CLBP patients, because of the fear, often exhibit prolonged inactivity and defensive, protective behavior [10, 11]. As a result, decrease in spinal mobility, muscle strength and cardiovascular endurance develops.

Klenerman et al. [12] found that fear avoidance is the most important predictor of chronicity in patients with back pain. Vlaeyen et al. [5,6] confirmed this in their work, showing that the fear of re-injury is a better predictor of the disability degree, than the intensity of pain and objective signs and symptoms of the condition themselves. They linked anxiety and fear with pain and spasm, successfully explaining the main problems in the treatment of CLBP.

Biopsychosocial Model for the Treatment of Patients with Chronic Pain

In the last twenty years, much has been said and written about the neuroanatomical pathways underlying CLBP, neuropsychological mechanisms that explain the complex feeling of pain, as well as the importance of both psychosocial factors in pain experience and individual response to nociceptive stimuli, and also how all of them can influence the success of the physical therapy. Pain is a complex perceptual experience that is influenced by psychosocial, social and biological factors. It is definite that chronic pain that have lasted for months, even years, will affect all aspects of the patient's functioning. Emotional, interpersonal, professional and physical life will be disrupted. With this in mind, patients suffering from chronic pain require attention and treatment not only of their physical, organic symptoms but also of a large number of factors that modulate and change the experience of pain and accompanying disability. To that end, Turk and Okifuji`s biopsychosocial model for the treatment of patients with chronic pain was created and accepted in 1999 [13]. Unlike one-dimensional models, both biomedical, which focus was only on the etiological and pathophysiological basis of the disease, and psychogenic, where pain and illness were presented as physical manifestations of a psychological disorder, biopsychosocial model combines purely mechanical and physiological processes with psychosocial influences that can cause, modify and prolong chronic pain. The biopsychosocial model presents illness as a dynamic and reciprocal interaction between biological, psychological, and sociocultural variables, which all shape a personal sense of pain [14]. This personal experience of pain is also in high percent, caused by the beliefs and convictions that each person acquires during their life. Based on his beliefs and the way patient accepts and experiences pain, he/she may choose to ignore the problem and continue working,

walking, socializing and maintaining similar level of previous activities, or may choose to leave work (temporarily or permanently), to cease all the activities and social interactions, accepting the role of the patient. Apart from personal prejudices and experiences, the influence of the environment, especially the spouse or other close family members, is not negligible. They can be promoters of both, healthy/active or unhealthy/passive response to pain and be that tongue on the scales that will judge if pain and illness will be dominating the patient's life. All of those aspects of the biopsychosocial model were the basis for the creation and application of CBT in the treatment of CLBP patients [15, 16].

The Aim

The aim of this study was to examine the effectiveness of individually designed exercises (IDE) and cognitive-behavioral treatment (CBT) in the therapy of patients with CLBP who have an over-protective posture and lack of natural body movements (avoiding activities that they think may reactivate or aggravate pain) and kinesiophobia (fear of movement).

Materials and Methods

A prospective randomized clinical study conducted at Clinic for physical medicine and rehabilitation UCC Niš, from March 2013 until December 2014, included 130 patients diagnosed with CLBP. The inclusion criteria were: over 18 years of age, pain in the lower back that lasted more than three months and clinical manifestation of lumbar instability combined with overprotective posture and lack of activities due to pathological fear of pain and body movement.

Prior to initiating therapy, subjects were informed of relevant research details. After the PMR specialist (physical medicine and rehabilitation specialist), had explained the reason and manner of their participation in the study, all the respondents signed the informed consent. .

The exclusion criteria from the study were: persons under 18 and over 75 years of age, bilateral radiculopathy or severe radiculopathy (myelopathy), complete stenosis of the spinal canal, recent injuries and surgical interventions in the lumbar region (in the last year), malignant and infectious diseases, and various organic diseases (lungs, heart, kidneys), which could limit the physical exertion, especially isometric muscle contractions.

After having taken a targeted anamnesis and in order to make an adequate diagnosis, a PMR specialist performed a clinical examination, which included assessment of posture, paravertebral muscle spasm, reflexes, the existence of radiculopathy and mobility of the lumbosacral spine. Specially designed kinesiological examination was also performed, including detection of: sign of instability, Gower's sign, reverse pelvic rhythm and excessive ligament laxity. Aberrant segmental mobility and pain provocation tests, such as instability test and passive extension test in prone position, were conducted. Strength and endurance of lumbar extensors and abdominal muscles were also measured.

Patients were randomly divided into four groups. They chose one of the three identical envelopes. Inside was a paper that assigned them to the protocol 1, 2 or 3. Control group 4 consisted of patients who could not come

Table 1 General information about the patients'groups

Group	G1		G2		G3		G4	
Gender								
female	23	(65.71%)	21	(60.00%)	20	(66.67%)	18	(60.00%)
male	12	(34.29%)	14	(40.00%)	10	(33.33%)	12	(40.00%)
Age (X±SD/(Me))	44.11 ± 10.53 (46.00)		50.91±11.33 (53.00)		48.03±12.44 (48.00)		46.23±12.83 (48.00)	
Education								
primary	0	(0.00%)	0	(0.00%)	3	(10.00%)	1	(3.33%))
high school	11	(31.43%)	13	(37.14%)	7	(23.33%)	12	(40.00%)
vocational	8	(22.86%)	12	(34.29%)	12	(40.00%)	6	(20.00%)
college	15	(42.86%)	10	(28.57%)	6	(20.00%)	10	(33.33%)
Master/PhD	1	(2.86%)	0	(0.00%)	2	(6.67%)	1	(3.33%)
Marital status								
married	15	(42.86%)	20	(57.14%)	21	(70.00%)	19	(63.33%)
live with	3	(8.57%)	1	(2.86%)	4	(13.33%)	1	(3.33%)
divorsed	8	(22.86%)	7	(20.00%)	3	(10.00%)	4	(13.33%)
widowers	2	(5.71%)	2	(5.71%)	1	(3.33%)	2	(6.67%)
single	7	(20.00%)	5	(14.29%)	1	(3.33%)	4	(13.33%)
Work								
active	13	(37.14%)	12	(34.29%)	14	(46.67%)	14	(46.67%)
sick leave	1	(2.86%)	2	(5.71%)	1	(3.33%)	4	(13.33%)
unemployed	15	(42.86%)	13	(37.14%)	8	(26.67%)	7	(23.33%)
housewife	1	(2.86%)	6	(17.14%)	5	(16.67%)	1	(3.33%)
student	3	(8.57%)	0	(0.00%)	1	(3.33%)	2	(6.67%)
retiree	2	(5.71%)	2	(5.71%)	1	(3.33%)	2	(6.67%)

to the therapy due to the distance from their place of residence or some other personal reasons.

Waddel's Fear Avoidance Beliefs Questionnaire (FABQ) and Tampa Scale of Kinesiophobia (TSK), were used for monitoring and evaluation. Patients completed them at the beginning, at the end of the therapy, and after 3 months.

Group 1 (G1; n = 35) had a combined IDE and CBT program. Group 2 (G2; n = 35) had IDE, without CBT. Group 3 (G3; n = 30) had standard group exercises for CLBP. Group 4 (G4; n = 30) was a control, patients did not have IDE nor CBT.

Individually designed kinesitherapy (IDE) was based on segmental spinal stabilization exercises. The exercise program was conducted in three phases:

Phase I – learning of joint isometric contraction of stabilizer muscles (formation and maintenance). At the beginning of the therapy, each patient learned how to form and maintain a protective lumbar muscle corset (by contracting the glutei muscles and retracting the lower abdominal wall) so that they could safely perform the dynamic part of the program.

Phase II – performing strengthening and stretching exercises. These are performed in a standing, sitting, kneeling and lying position. The obligatory set of exercises consists of elevation of the pelvic girdle (bridging), abdominal training, stretching of the back by extension and flexion of the back muscles (from a four-pronged position), postural correction and posterior inclination of the pelvic girdle (hook-lying). The program also contains a set of exercises on unstable support, which aim to activate the neural subunit of the dynamic lumbar segment and improve proprioception, coordination and balance.

Phase III – based on the incorporation of learned isometric contractions and active exercises into the activities of everyday life. It contains a simulation of the movements that patients most often perform in the work and social environment. The program also includes breathing exercises and ergonomic counseling.

Kinesitherapy treatment was individually composed and carried out in groups of a maximum 5 patients, for 3 weeks; altogether 15 therapeutic treatments, lasting from 15 to 30 minutes each (dosed-progressive), or 20 minutes (standard program for CLBP).

Cognitive-behavioral treatment (CBT) accompanied exercises. It was performed according to the principles of Beck's cognitive therapy for the treatment of patients with chronic pain [17]. In the practical implementation of the program, a therapeutic guide by Otis was used [18].

The program consisted of 12 sessions: 1. Initial interview and goal setting; 2. Education about chronic pain syndrome; 3. Theory of pain and diaphragmatic breathing; 4. Progressive muscle relaxation and imaging techniques; 5. Automatic thoughts of pain; 6. Cognitive restructuring; 7. Stress management; 8. Timing activities in order to avoid provoked pain; 9. Planning enjoyable activities; 10. Anger management; 11. Sleep hygiene; 12. Relapse prevention and control schedule .

Before the first session, patients were asked to complete the following forms:

- Pain Interview - an interview that includes: basic information about the patient; details about the beginning of the illness, intensity and character of pain, psychosocial anamnesis - schooling, marital status, recreational activities, etc [18].
- Goal Setting Worksheets - specific CBT goals (1-5), with the possibility of weekly monitoring. In the table extension, patients are asked to write down what would be the minimum, moderate or complete meeting of previously set goals [18].

In addition to these standard questionnaires, a large number of tables and worksheets were used during the therapy, in order to actively involve patients in their own therapeutic treatment.

Cognitive-behavioral treatment consisted of 12 sessions which were performed in groups of max 10 patients, 2 times a week, with the treatment duration of 90 minutes.

Statistical Analysis

The data collected from the questionnaires were classified and stored in the MS Office Excel database. Statistical analysis of the data was performed with the software package SPSS 15.0. The results of the data analysis were presented in a table. Continuous variables were represented by mean values and standard deviations (X±SD) and medians (Me) as a measure of central tendency. Categorical variables erre given as absolute numbers and percentages. The examination of the normality of the distribution of continuous variables was tested, depending on the sample size, by the Kolmogorov-Smirnov or Shapiro-Wilk test. Comparison of the values of continuous variables between two control periods, depending on the normality of the distribution, was performed by Student's t-test of dependent samples (in case of normal distribution) or Wilcoxon's Signed Ranks test (when distribution deviates from normal). The effect of the therapy in individual groups was determined by the Cohen's d parameter for dependent samples, which was calculated as: $d=\Delta X/\Delta SD$, where ΔX represents the mean of the changes and ΔSD represents their standard deviation. The limit values of Cohen's d are 0.2, 0.5 and 0.8, which differentiate between small, average and large effect of the therapy. Comparison of the age of the respondents between the groups was performed by ANOVA analysis and the consequent Post Hawk analysis. Testing the proportion of categorical variables between groups was examined by Pearson's χ^2 test or appropriate modifications of this test.

Results

Fear avoidance behaviors in CLBP patients were observed from two equally important angles: fear of physical activity and fear of work related activities, using Waddel's Fear Avoidance Belief Questionnaire (FABQ). Regarding patients fear of any physical activity, it was noted that immediately after the therapy and also after three months, all the

scores were statistically significantly lower (better) in groups G1 and G2 ($p < 0.001$), and higher in G3 ($p < 0.05$) and G4 ($p < 0.01$).

The effect of the therapy, based on Cohen's d value, was positive and very high in G1 and G2, and negative and completely insignificant in groups G3 and G4. Even after 3 months of therapy, the effect remained high and positive in G1 and G2, and negative, but now lower in intensity, in G3 and G4. It should be noted that in relation to the condition before therapy, the effect was more pronounced 3 months after therapy than immediately after, but only in group G1, while in the other three groups the effect was somewhat smaller.

Using the previously explained instructions for scoring in the examined groups, the following values of the scale for avoiding work (professional) activities were obtained based on the FABQ questionnaire: before, after and 3 months after the therapy. Scale scores have a higher value for greater avoidance of work (professional) activities.

Based on the data in table 3, it is evident that in relation to the scores before therapy, there was a statistically significant decrease of the measured values only in groups G1 and G2 ($p < 0.001$), right after and 3 months after the therapy. In G3 and G4, the decline in the rate of avoidance of work -related activities was very small. Comparing groups G1 and G2, it was noted that scores after 3 months of therapy in G1 was lower than one immediately after therapy, while in G2 it was almost identical, which may speak in favor of more lasting effects of therapy used in G1. The therapy effect on reducing the avoidance of work-related activities was of a medium intensity immediately after therapy and after 3 months, while the effect in G3 and G4 was practically non-existent. This indicates that only implemented therapies in groups G1 (IDE+CBT) and G2 (IDE) had a positive impact on reducing fear of work-related activities.

Kinesiophobia in CLBP patients was evaluated by Tampa Scale of Kinesiophobia (TSK).

Based on the scoring instructions for this scale, higher scores indicate higher kinesiophobia, and their values, based on the TSK, taken before, after and 3 months after therapy are given in table 4.

Table 2 Waddel's Fear Avoidance Belief Questionnaire (FABQ): physical activities

	G1			G2			G3			G4		
before th	16.26 ±	4.74	(18.00)	16.34 ±	5.96	(17.00)	14.57 ±	5.61	(15.00)	15.80 ±	4.10	(16.00)
after th	11.49 ±	4.88	***(12.00)	13.49 ±	4.90	***(14.00)	15.20 ±	5.83	*(16.00)	16.40 ±	4.44	**(17.00)
after 3 m	10.74 ±	5.01	***(11.00)	13.40 ±	4.89	***(14.00)	15.10 ±	5.89	*(15.50)	16.33 ±	4.57	*(16.50)
aft/bef	4.77 ±	3.83	(4.00)	2.86 ±	2.14	(3.00)	-0.63 ±	1.27	(0.00)	-0.60 ±	1.16	(-0.50)
3m a/b	5.51 ±	4.02	(5.00)	2.94 ±	2.39	(3.00)	-0.53 ±	1.14	(0.00)	-0.53 ±	1.22	(0.00)
d aft/bef		1.25			1.33			-0.50			-0.52	
d 3 a/b		1.37			1.23			-0.47			-0.44	

* – $p<0.05$, ** – $p<0.01$, *** – $p<0.001$

^ Changes in scores compared to the period before Th (X ±SD, Me) and the achieved therapy effect (Kohens'd)

Table 3 Waddel's Fear Avoidance Belief Questionnaire (FABQ): work related activities

	G1			G2			G3			G4		
before th	17.51 ±	10.40	(16.00)	14.60 ±	11.75	(14.00)	17.10 ±	13.34	(16.00)	18.23 ±	14.71	(17.00)
after th	15.20 ±	9.46	***(12.00)	11.66 ±	10.16	***(11.00)	16.93 ±	12.92	(16.00)	18.00 ±	14.64	(16.00)
after 3 m	14.57 ±	8.91	***(11.00)	11.69 ±	10.23	***(11.00)	16.80 ±	12.69	(17.00)	18.00 ±	14.56	(16.00)
after/bef	2.31 ±	3.69	(3.00)	2.94 ±	4.47	(1.00)	0.17 ±	1.44	(0.00)	0.23 ±	1.55	(0.00)
3m a/b	2.94 ±	4.19	(4.00)	2.91 ±	4.56	(1.00)	0.30 ±	1.93	(0.00)	0.23 ±	1.55	(0.00)
d aft/bef		0.63			0.66			0.12			0.15	
d 3m a/b		0.70			0.64			0.16			0.15	

*** – $p<0.001$

^ Changes in scores compared to the period before Th (X ±SD, Me) and the achieved therapy effect (Kohens'd)

Table 4 Tampa scale of Kinesiophobia (TSK)

	G1			G2			G3			G4		
before th	39.9 ±	8.01	(40.00)	44.09 ±	6.92	(43.00)	43.03 ±	5.29	(43.50)	43.70 ±	4.53	(43.00)
after th	34.31 ±	6.37	***(33.00)	40.46 ±	5.98	***(40.00)	43.10 ±	6.19	(43.50)	43.83 ±	5.41	(44.00)
after 3 m	34.26 ±	6.40	***(34.00)	40.11 ±	6.15	***(39.00)	42.87 ±	6.07	(44.00)	43.53 ±	5.31	(44.50)
aft/bef	5.63 ±	4.56	(5.00)	3.63 ±	3.98	(4.00)	-0.07 ±	2.30	(0.00)	-0.13 ±	2.33	(0.00)
3m a/b	5.69 ±	4.55	(5.00)	3.97 ±	4.03	(3.00)	0.17 ±	2.21	(0.00)	0.17 ±	2.23	(1.00)
d a/b		1.23			0.91			-0.03			-0.06	
d 3ma/b		1.25			0.99			0.08			0.07	

*** – $p<0.001$

^ Changes in scores compared to the period before Th (X ±SD, Me) and the achieved therapy effect (Kohens'd)

Compared to pre-treatment values, significantly lower TSK scores were obtained, after therapy and 3 months after, in G1 and G2 ($p < 0.001$), while in G3 and G4 there were no statistically significant changes. The effect of therapy on the reduction of kinesiophobia was positive and high in groups G1 ($d = 1.23$ and 1.25) and G2 ($d = 0.91$ and 0.99), while it was completely insignificant in the other two groups. The intensity of the therapy effect was higher in the G1 group which, in addition to the experimental kinesitherapy treatment, also had cognitive-behavioral therapy.

The above mentioned results showed that in patients with CLBP, which presented a fear-induce avoidance behavior regarding physical and work-related activities, gradual introduction into kinesitherapy treatment had the greatest effect on pain, functionality and overall quality of life. Step by step exposure to the fearful exertions and movements induces the change in a patient's beliefs and preconceptions regarding activity and pain. The fear of movement and self imposed contrived body positions, held by a patient in order to avoid any unnecessary and potentially painful movements can be very difficult to overcome using only dosed physical activity. With this in mind, we can conclude that the necessity of CBT in the CLBP treatment is unquestionable.

Discussion

This study examined the effects of a combined individually designed and implemented kinesitherapy treatment for low back pain in combination with cognitive-behavioral therapy, on fear avoidance behavior and kinesiophobia in CLBP patients. Individually assembled and conducted kinesitherapy treatment, which consists of stabilization, strengthening and stretching exercises combined together, represents the basic therapy protocol for the patient with chronic low back pain [19]. On the other hand, the role of CBT had also proven to be very important. The various authors, over the years, compared the effect of CBT in relation to different kinesitherapy programs and also in relation to the combined exercises with CBT protocols [20]. Many of those studies showed that having CBT as a part of a treatment protocol, either alone or with exercises, significantly reduces not only pain itself, but also a fear of pain and kinesiophobia [21, 22].

In order to monitor the results of the treatment in this study, two very useful questionnaires were used: Waddel's Fear Avoidance Belief Questionnaire (FABQ) and Tampa Scale of Kinesiophobia (TSK). Swinkels-Meewissea et al. confirmed their validity for monitoring fear of pain in CLBP patients [23]. Studies by George et al. and Johnson et al. showed that these questionnaires could also be used as a predictive tool to isolate patients who may benefit from CBT, even on their first doctors' visit, before they developed chronic pain syndrome and all the accompanying physical and psychological complications [24, 25]. Their studies showed that patients who had a more pronounced fear of pain and physical activities before therapy, showed better results after CBT, than those who initially had a lower score of FABQ and TSK. In this way, we can predict whether the acute pain will become chronic and whether to include patients in CBT or not. This is very important, because this kind of treatment requires a lot of time and commitment from the patient and the physician both.

Leeuw et al. [1] in their study examined the effect of CBT on the treatment of CLBP patients that had had a fear of pain-related activities and found that CBT showed promising results in reducing these ailments, and thus indirectly affected the course, duration and outcome of CLBP. Vlaeyen and Morley [26] demonstrated in 2005 their idea that each patient should receive adequate treatment according to their characteristics, showing this in the group of patients who were treated only by general practitioners. They proved that the CLBP patients with previously recorded symptoms of catastrophizing and somatization did not have a positive outcome of treatment with classical methods. Jellema et al. [27] confirmed this statement when they included minimal cognitive interventions in the treatment plan of patients with CLBP. Those interventions were focused only on the recorded risk factors of chronicity (fear of movement, somatization, inadequate activities and body positions, exaggeration, etc.). Patients treated in this way had significantly better results.

In recent years, CBT has deservedly taken its permanent place in the CLBP treatment protocol. Numerous authors: Although Burns [28], Smeets [29], Spinhoven [30], Sullivan and Stanish [31], O'Sullivan [32] and many others applied different CBT techniques, they got positive results. The reduction of catastrophic symptoms and fear of pain and movement in CLBP patients was what all of these studies had in common. The abovementioned showed that not only classical CBT but also short targeted CBT interventions can have significant positive effects on pain and functionality in CLBP patient's treatment.

After all this has been said, it is clear that the fear of movement and accompanying maladaptive behaviors, presents important part of CLBP and plays a major role in selecting adequate therapy. What remains is a dilemma how to use this knowledge in preventing chronicity and fear avoidance behavior and kinesiophobia from even happening. After all this, what we can say for sure, is that in acute episodes of low back pain, these symptoms are often absent and there is no reason for using CBT. Also, in cases of subacute pain, some authors suggest short educations instead of a complete CBT program [33, 34, 35]. On the other hand complexity of CLBP is a clear indication for inclusion of CBT in treatment protocol. However, it is not still clear whether we can change future behavior of the patients altogether.if we only influence their thoughts and beliefs, explaining them that the fear of movement is unfounded and inadequate response. Additional research is needed in this direction. One of the certain benefits of this and similar studies is the fact that FABQ and TSK could be used as a prediction tool of chronicity in patients who are still in an acute or subacute stage of illness. In patients with a confirmed risk of prolonged and complicated low back pain, CBT can be used as a preventive measure so that patients do not develop chronic pain syndrome.

Conclusion

Combined IDE and CBT have had a positive effect on the frequency and intensity of pathological physical and psychosocial symptoms: fear avoidance behavior and excessive fear of movement (kinesiophobia), in CLBP patients. A statistically significant improvement was recorded both immediately after the therapy and three months later, which indicates the permanence of the effect of this experimental treatment.

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Original Scientific Article

AGRYPON POLYXENAE (SZÉPLIGETI, 1899) (HYMENOPTERA: ICHNEUMONIDAE: ANOMALONINAE); NEWLY RECORDED PARASITOID OF *ZERYNTHIA POLYXENA* (DENIS & SCHIFFERMÜLLER, 1775) (LEPIDOPTERA: PAPILIONIDAE: PARNASSIINAE) IN THE FAUNA OF SERBIA

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Abstract. *Ichneumonid Agrypon polyxenae is a solitary larval-pupal endoparasitoid developing within the caterpillars of the genus Zerynthia and distributed in the western Palearctic. Z. polyxena is the most common host, with its caterpillars feeding on highly poisonous Aristolochia species. Here we report A. polyxenae parasitising Z. polyxena for the first time in Serbia and provide a short species description. This parasitoid has also been recorded on Z. rumina in Spain. We suggest some evidence for its presence in the newly described Z. cassandra in Italy*.

Key words: *festoons, caterpillars, larval-pupal parasitoid, Aristolochia*

Introduction

Parasitic wasp *Agrypon polyxenae* (Szépligeti, 1899) is a slender body species and it is one of the larger members of Ichneumonidae family. This elegant wasp has been recorded as a solitary koinobiont endoparasitoid of caterpillars of the butterfly genus *Zerynthia* [1]. Its primarily host is the southern festoon, *Z. polyxena* (Denis & Schiffermüller, 1775). Since *A. polyxenae* parasitizes economically unimportant hosts, there is not much literature on its biology. Females are known to oviposit in mature *Zerynthia* caterpillars, where the parasitoid larva develops. The emergence of parasitoid imago occurs in the pupal stage of the host. Therefore, this kind of parasitism is defined as a larval-pupal parasitoid. Following the biology of its host, *A. polyxenae* has one generation per year and overwinters inside the host pupa. The integument of the pupa is known to be extremely strong and resistant to harsh weather conditions during a prolonged period, which can last over ten months [1]. Due to such strong skin, the adults of *A. polyxenae* chew the irregular exit hole on the integument of the host's pupa in its anterior dorsal part [1].

Being distributed in a large portion of the western Palearctic, *Z. polyxena* is the most common host of *A. polyxenae* [2]. However, there is solid evidence that it also parasitizes the Spanish festoon, *Z. rumina* (Linnaeus, 1758) [3, 4]. Although classified as LC (Least Concern), populations of *Z. polyxena* are undoubtedly in decline throughout Europe, and therefore strictly protected in Serbia and listed in the Habitats Directive 92/43 EEC of the European Union [5]. The taxonomy of the species is rather complex [6], as the great variability of the wing pattern triggered the description of over 30 subspecies and forms, where most of them belong to local populations. The very short flight period, and the specific food plant/microhabitat demands, make *Z. polyxena* convenient for further exploration of the post-glacial speciation [7]. It usually occurs in habitats lower than 900 m above sea level, but the altitude range may vary between 0 and 1700 m a.s.l. [8, 9]. The species occupies wetland areas close to water streams, riparian woodlands, abandoned agricultural land, roadsides, and urban areas, usually in patchy populations. This stenophagous caterpillar feeds on highly poisonous *Aristolochia* species (Piperales: Aristolochiaceae), namely *A. clematitis* L., *A. pallida* Willd., *A. pistolochia* L., *A. pontica* Lam., *A. rotunda* L. and *A. sicula* Tineo, with different local preferences [10]. Depending on the geographic region, the butterfly can be locally monophagous [11]. Along with several other plant genera from the family Aristolochiaceae, *Aristolochia* species are highly toxic and even carcinogenic to humans and responsible for the environmentally driven Balkan endemic nephropathy (BEN) [12].

In this paper, we report *A. polyxenae* for the first time in Serbia and give a more detailed species description and notes on its record. Furthermore, we discussed *Z. cassandra* (Geyer, 1828) as a potential host of *A. polyxenae*.

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Materials and Methods

Caterpillars of *Z. polyxena*, mainly 4th and 5th instar, were found on *A. clematitis*, usually on the leaf underside. In total, 56 caterpillars were collected. Individuals were put into plastic containers of approximately 0.5 litres of volume. The caterpillars were fed with birthwort leaves *ad libitum* in expectation of parasitoid appearance. All obtained parasitoid individuals were pinned and mounted on cardboards and prepared specimens were photographed using Nikon stereomicroscope (SMZ 745T). The distribution of *A. polyxenae* was compiled according to TaxaPad databases [13].

Results

Only four parasitoids emerged from *Z. polyxena* pupae. All of them belonging to *A. polyxenae*. The analysed material includes: 3♂ Idvor (45°11′18″ N; 20°30′47″ E), 15.09.2019, emerged on 04.05.2020, leg. A. Husarik; 1♀ Kladovo (44°36′24″ N; 22°36′47″ E), 05.05.2021, leg A. Trajković. Photographs of both male and female *A. polyxenae* habitus as well as selected body details were presented in Figure 1 (A-H). Also, the mummified pupa of *Z. polyxena* from which the parasitoid emerged was presented (Fig. 1I). The exit hole is partly visible on the dorsal anterior part of the empty mummy.

Short description

Female: body length 13.5 mm; head black with yellow frons; mandible yellow; ocelii black; antennae black, 51-segmented, first flagellar segment 2.5 x longer than the second; mesosoma entirely black covered with white dense setae; mesoscutum shiny, punctuated; both pair of wings hyaline; coxae and trochanters of all legs black, first and second pair of legs yellowish, hind legs brown; metasoma elongated, twice as long as the wings; petiole brown, slender rod-shaped; metasomal segments 2-4 brown, the rest black; proximal part of ovipositor black, distal part brown, length of ovipositor almost equal to hind tibia.
Male: body length 15-17 mm; general morphology and coloration as in female. All legs brown.

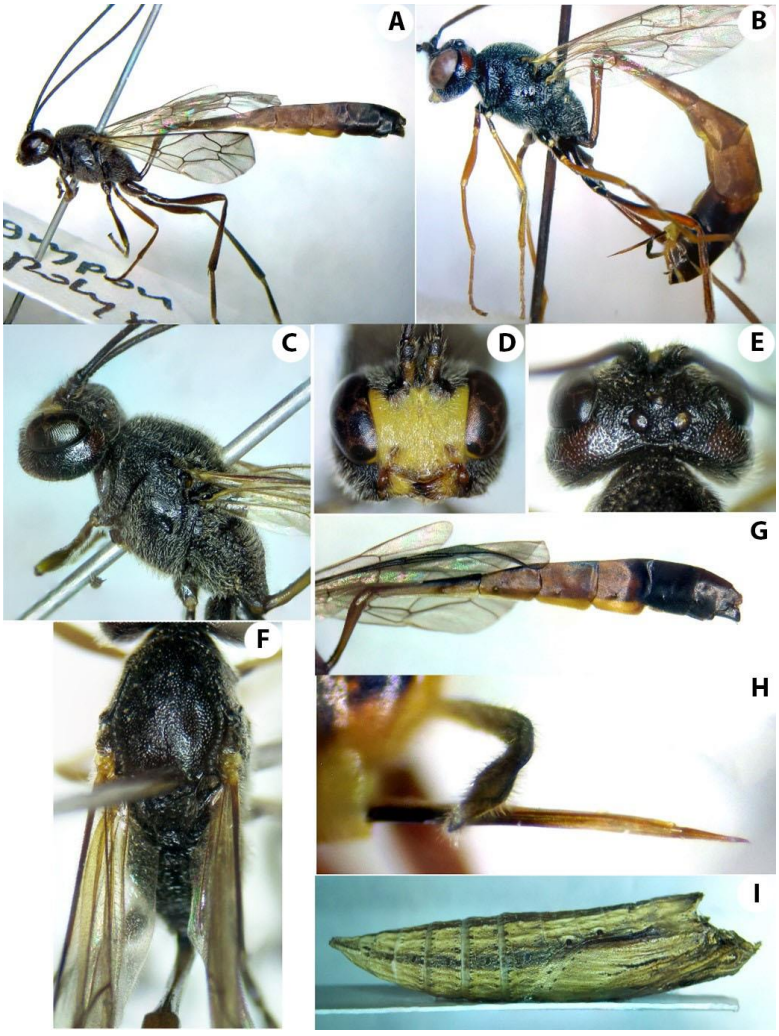

Fig. 1 General morphology of *Agrypon polyxenae*: A – male, B – female, C – lateral aspect of head and mesosoma (male), D – frontal aspect of the head (male), E – temporal aspect of the head (male), F – dorsal aspect of mesosoma (male), G – lateral aspect of metasoma (male), H – ovipositor, I – mummified pupa of *Zerynthia polyxena/*

Distribution: Austria, Bulgaria, Czech Republic, Germany, Hungary, Italy, Romania, Russia (Astrakhanskaya Oblast, Dagestanskaya Respublika, Samarskaya Oblast), Serbia, Spain, Ukraine.

Discussion

The flight period of *Zygaena polyxena* is only a few weeks long, in Serbia it occurs usually between late-March and mid-May, depending on the altitude (Biologer Community in Serbia 2018; Popović et al., 2020). On the territory of Serbia, the primary host plant for this butterfly is *Aristolochia clematitis*, a very abundant species, especially in ruderal areas. *A. pallida* has also been reported sporadically as a feeding plant, primarily in the regions with higher altitudes [14, 15]. Since *A. pallida* and *A. lutea* are morphologically very similar and given the distribution of *A. lutea* on the territory of Serbia [16], it is more likely that *Z. polyxena* was found on *A. lutea* in some reports due to misidentification of *Aristolochia* species. It should be noted here that the taxon *Z. cassandra* was separated from the *Z. polyxena* complex and raised to the species level. This species inhabits Italy [17] and it is associated with *Aristolochia rotunda* and *A. lutea* [18, 19]. Overlapping distribution of *Z. cassandra* and *A. polyxenae* indicates that *A. polyxenae* parasitizes a total of three species of *Zerynthia*, for which additional research is needed.

It is important to mention that in the literature, *Euproctis chrysorrhoea* (L.) (Erebidae) is also registered as a host for *A. polyxenae* [20]. However, this information is quite unreliable because it is unaccompanied by other sources and very likely to be an erroneous, less possible misidentification.

Conclusion

Analysed material in this study comes from the caterpillar samples collected exclusively from *A. clematitis*. Despite dozens of collected specimens, the percentage of observed parasitism was apparently very low. However, we cannot state this as a fact merely due to statistical reasons. The scarce literature data on *A. polyxenae* has impaired comparison of our data and no significant conclusion can be extrapolated. Also, surveying online databases did not show any data on *A. polyxenae* occurrence in the territory of Serbia.

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