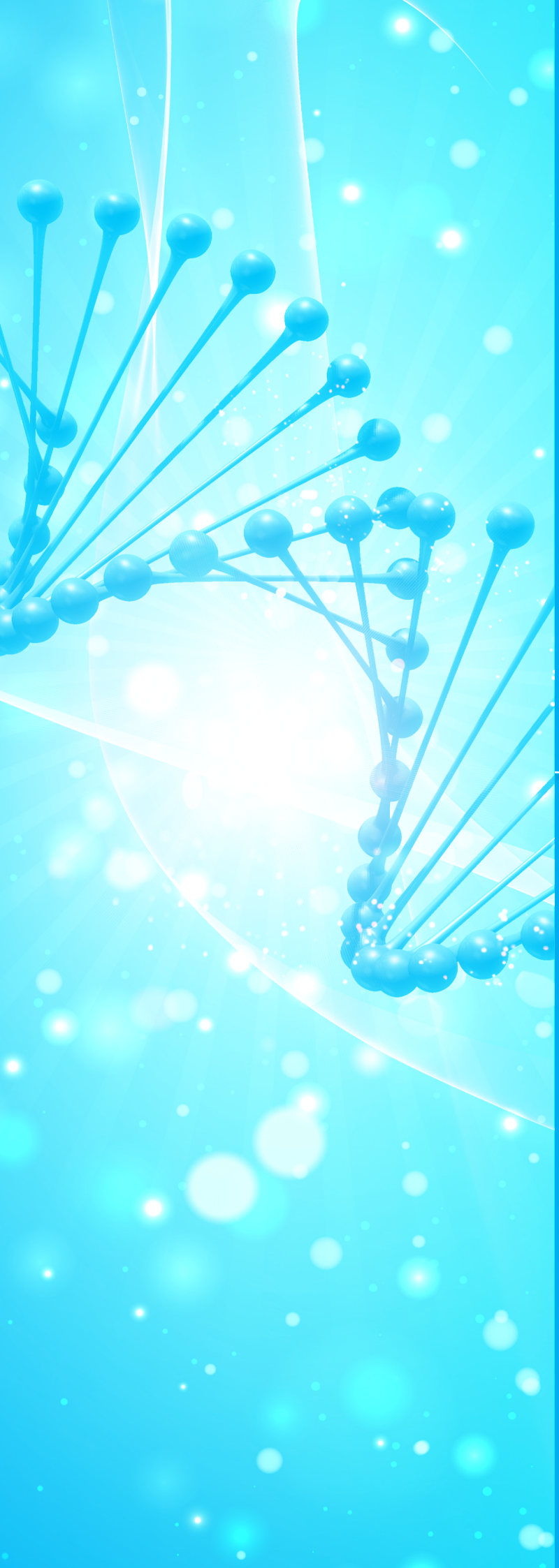

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GOST UREDNIK

Prof. Valentina Arsić Arsenijević, MD, Msc, PhD, FECMM je osnivač i rukovodilac Nacionalne referentne laboratorije za medicinsku mikologiju i redovni profesor na Medicinskom fakultetu Univerziteta u Beogradu. Stekla je akademska zvanja i zvanja specijalista mikrobiologije i subspecijalista mikologije na istom fakultetu. Gostujući profesor i istraživač na Univerzitetu u Ljubljani (Slovenija), Univerzitetu Karolinska (Švedska), Univerzitetu Insbruk (Austrija) i Univerzitetu Sasari (Italija).

Osnivač je Univerzitetske biotehnološke *spin-out* kompanije (*MyCoMedlab*) i osnivač i predsednik Društva medicinskih mikologa Srbije. Predstavlja Srbiju kao ambasador Globalnog akcionog fonda za gljivične infekcije (GAFFI), ambasador je kongresnog biroa Srbije i saradnik u grupi Globalno opterećenje bolestima (GBD), predstavlja Društvo medicinskih mikologa Srbije u Evropskom drustvu za kliničku mikrobiologiju i infektivne bolesti (ESCMID) i studijskoj grupi za proučavanje gljivičnih infekcija ovog društva (EFISG), Međunarodnom udruženju za humanu i animalnu mikologiju (ISHAM) i u Evropskoj konfederaciji medicinske mikologije (ECMM). Bila je lokalni organizator evropskog kongresa „*Trends in Medical Mycology*" – 2017 (TIMM 8) i predsednik izvršnog odbora, kao i član predsedništva ECMM (2014-2017). Bila je predavač po pozivu na brojnim nacionalnim i međunarodnim konferencijama.

Prof. Arsić Arsenijević objavila je više od 200 članaka u prestižnim časopisima (uključujući *Lancet*) i knjige za studente i doktore medicine. Recenzent je u preko 40 medicinskih naučnih časopisa, uključujući *Mycoses*. Osnovala je sajtove *www.mikologija.org.rs* i *www.dtfd.org* koji imaju 600.000 posetilaca godišnje. Vodila je i učestvovala u više od 20 nacionalnih i međunarodnih projekata. Trenutno vodi projekat „Rana laboratorijska detekcija biomarkera i značaj za ishod invazivnih gljivičnih infekcija (IFI) u Srbiji". Fokus njenih istraživanja i publikacija su respiratorne infekcije, a poseban fokus je laboratorijska dijagostika invazivnih gljivičnih infekcija, testiranje antifungalne osetljivosti, lokalna epidemiologija gljivičnih infekcija, modelovanje bioloških procesa, inovativni pristup personalizovanoj medicinskoj mikologiji, i istorija srpske medicine. Njeni radovi su citirani više od 13.400 puta (sve), sa H-indeksom 21 (sve); i 10-indeksom 39 (sve) (Google Scholar); RG skor je 37.04, H-indeks 22 (sve) (Research Gate). Ukupan broj publikacija *in extenso* iznosi 114 (67 u JCR) sa ukupnim impakt faktorom publikacija 546.521.

GUEST EDITOR

Prof. Valentina Arsic Arsenijevic, MD, Msc, PhD, FECMM is founder and head of the National Reference Laboratory for Medical Mycology and Full university professor at Faculty of Medicine, University of Belgrade, Belgrade, Serbia. She received her academy degrees, Microbiology specialist, and Mycology subspecialist degrees from the same University. She has been a visitor scientist, visiting professor or a research fellowship in University of Ljubljana (Slovenia), Karolinska University (Sweden), University of Innsbruck (Austria), and University of Sassari (Italy).

She is founder of University spinout biotechnology company (MyCoMedlab) and founder and president of Serbian Society of Medical Mycology (SSMM). She serves as Global Action Fund for Fungal Infections (GAFFI) ambassador and Serbian congress ambassador, and collaborator of Global Burden Diseases (GBD) group. She represents SSMM at European Society of Clinical Microbiology and Infectious Diseases (ESCMID), and Fungal Infection Study Group (EFISG), International Society of Human and Animal Mycology (ISHAM), and European Confederation of Medical Mycology (ECMM). She was local organizer of Trends in Medical Mycology – 2017 (TIMM 8) and president of the Executive Board, as well as member of ECMM board (treasurer 2014-2017). She has been an invited speaker and chair in several national and international conferences.

Prof. Arsic Arsenijevic published more than 200 articles in prestigious journals (including the Lancet) or books for University students and medical doctors. She has been a reviewer for more than 40 medical scientific journals, including Mycoses. She established websites (www.mikologija.org.rs and www.dtfd.org) which attracts about 600,000 visitors yearly. She had led and participated in over 20 national and international scientific projects. Currently she leads project "Early laboratory biomarker detection and significance for outcome of invasive fungal infections (IFI) in Serbia". Her research and publications focus are respiratory infections and laboratory diagnosis of invasive fungal infections, antifungal susceptibility testing, local fungal epidemiology and resistance, modeling of mycological process, innovative approach in personalized medical mycology, and history of Serbian medicine. Her work has been cited over 13.400 (all), H-index 21 (all); i10-index 39 (all) (Google Scholar); RG score 37.04, H-index 22 (all) (Research Gate). She has total number of publications *in extenso* 114 (67 in JCR) with total impact factor of publications: 546.521.

TEMATSKI BROJ ČASOPISA POD NAZIVOM „JAVNOZDRAVSTVENI, KLINIČKI, DIJAGNOSTIČKI, TERAPIJSKI I EKONOMSKI ASPEKTI KOVID-19“

Beograd, 22. septembar 2020. godine

UVODNA REČ GOSTA UREDNIKA

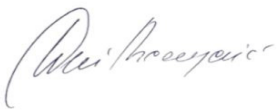
Kovid-19 pandemija je potresla čovečanstvo, sa posledicima čiji će se bilans svoditi mesecima. Već sada svet plaća nemerljivu cenu zbog nepoznavanja i potcenjivanja infektivnih agenasa i njihovog medicinskog i javnog značaja. Zato je važno da se snažnije udruže sve grane medicine, preventivne, dijagnostičke i kliničke, kako bi multidisciplinarnim pristupom spremno odgovorile na ovaj izazov, ili neki sledeći.

U okviru aktuelne pandemije, a u cilju kontrole širenja SARS-CoV-2, ključno je bilo pravovremeno otkrivanje virusa i rezervoara, kao i brzo i precizno testiranje, izveštavanje i reagovanje u svim sumnjivim slučajevima. Takođe, baza kliničkih i laboratorijskih podataka je od ogromne važnosti za svaku zemlju, te je veliki značaj dugoročnih aktivnosti. Kod nas, takvu ulogu ima Nacionalna referentna laboratorija za virusne respiratorne infekcije „Instituta za virusologiju, vakcine i serume Torlak“, kao i mreža laboratorija koje funkcionišu u okviru Globalnog sistema za nadzor i reagovanje na grip.

Radeći uglavnom „nevidljivo“, ove laboratorije su bile i ostale vitalna karika u lancu aktivnosti potrebnih da se što veći deo nacije sačuva od Kovid-19 bolesti, a njihova dobra priprema je predstavljala prvu liniju odbrane od novog koronavirusa. Ove laboratorije ne čini samo tehnologija, oprema i prostor, već i odgovarajući ljudi i sistem za kontrolu i primenu laboratorijskih procesa i standarda neophodnih za pravovremenu i tačnu dijagnozu, u čemu edukacija i razmena znanja i iskustva ima najveći značaj. Zbog toga je uloga autora radova ovog specijalnog broja časopisa „**Zdravstvena zaštita**“ od neprocenjivog značaja, a njihovo iskustvo naš zdravstveni sistem treba da ceni, a savete i preporuke da uvaži.

Zahvaljujem se uredništvu časopisa „**Zdravstvena zaštita**“ što je na ovaj način promovisalo njihovu ulogu i omogućilo celokupnoj zdravstvenoj struci Republike Srbije da dobije ovako značajne informacije.

Prof. dr Valentina Arsić Arsenijević



THEMATIC ISSUE TITLED "THE PUBLIC HEALTH, CLINICAL, DIAGNOSTIC, THERAPEUTIC AND ECONOMIC ASPECTS OF COVID-19"

Belgrade, September 22nd, 2020

AN INTRODUCTORY WORD BY THE GUEST EDITOR

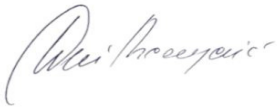
The Covid-19 pandemic has shaken the mankind, and it will take time to perceive all the repercussions. The world has already paid the immeasurable price for the ignorance and underestimation of infectious agents and their medical and public significance. Therefore, it is important to strongly unite all branches of medicine, preventive, diagnostic and clinical, in order to be ready to respond with this multidisciplinary approach to this challenge, or the following ones.

In the current pandemic, timely discovery of the virus and reservoirs has been of key importance for the control of SARS-CoV-2 spreading, as well as rapid and precise testing, reporting and reacting in all suspicious cases. Also, the base of clinical and laboratory data is of utmost importance for all countries, especially in terms of long-term activities. In our country, the National Reference Laboratory for Viral Respiratory Infections of the Institute of Virology, Vaccines and Sera "Torlak" has such a role, as well as the network of laboratories, which function within the Global Influenza Surveillance and Response System.

By working mostly "invisibly", these laboratories have remained the vital ring in the chain of activities necessary to protect the nation from the Covid-19 disease as much as possible, and their good preparation presented the first line of defense from the novel corona virus. Technology, equipment and space are not the only things that make these laboratories. These laboratories are made up of people and the system for the control and application of laboratory processes and standards necessary for the timely and precise diagnosis, in which education and exchange of knowledge and experience have the utmost importance. Therefore, the role of authors of this special issue of the journal "**Health Care**" is invaluable, and their experience should be respected by the health care system, while their advice and recommendations should be taken into consideration.

I wish to express my gratitude to the editorial board of the journal "**Health Care**" because they promoted their role in this way and made it possible for the whole health profession of the Republic of Serbia to get such important information.

Prof. Valentina Arsic Arsenijevic, PhD



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UTICAJ SOCIODEMOGRAFSKIH FAKTORA NA RAZVOJ ANKSIOZNOSTI U TOKU PANDEMIJE KOVID-19

Marijana Jandrić-Kočić[1], Snežana Knežević[2]

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SAŽETAK

Uvod/Cilj: Pandemija Kovid-19 predstavlja ozbiljnu pretnju globalnom mentalnom zdravlju. Strah od smrti, socijalno distanciranje, ekonomski problemi, promene u obrazovanom sistemu i prestanak uobičajenog svakodnevnog funkcionisanja indukuju psihijatrijske poremećaje, u prvom redu anksioznost. Cilj istraživanja je bio da se utvrditi uticaj sociodemografskih faktora na razvoj anksioznosti u toku pandemije Kovid-19.
Metode: Studijom preseka, tokom dva meseca, bilo je obuhvaćeno 200 osoba bez simptoma Kovida-19, 60,0% žena i 40,0% muškaraca, prosečne starosti 51,0 ± 9,2 godina. Sto osoba se javilo u ambulantu porodične medicine Doma zdravlja Krupa na Uni zbog pregleda ili administrativnih razloga, a drugih sto je bilo u njihovoj pratnji. Pored opšteg upitnika za prikupljanje podataka, korišćen je Upitnik za samoprocenu intenziteta simptoma generalizovanog anksioznog poremećaja (engl. *Generalized Anxiety Disorder-7*, GAD-7). U statističkoj analizi podataka korišćeni su χ^2-test i *Fisher*-ov test.
Rezultati: Simptome anksioznosti imalo je 52,5% ispitanika uključenih u ovu studiju preseka, i to 35,0% blage, 15,5% umerene i 2,0% ozbiljne. Anksioznost je nešto češće bila kod muškarca (56,2%) nego žena (50,0%), kao i kod starijih (60-79 godina) (92,6%) nego mlađih ispitanika (12,5% za uzrast 20-39 i 44,7% za uzrast 40-59 godina). Osobe sa nižim stepenom obrazovanja (44,5% ispitanika sa osnovnom i 46,6% sa srednjom školom) su češće bile sa simptomima anksioznosti, nego osobe sa višim stepenom obrazovanja (33,3%), zaposleni (69,5%) u odnosu na nezaposlene (43,0%), i osobe koje žive u urbanoj (80,8%) nego ruralnoj sredini (42,6%). Simptomi anksioznosti zabeleženi su kod 51,3% ispitanika koji su bili u braku ili vanbračnoj zajednici i kod 56,8% ispitanika koji nisu bili u braku ili vanbračnoj zajednici. Osobe sa simptomima anksioznosti su značajno češće bile uzrasta 60-79 godina nego osobe bez anksioznosti. Između ovih grupa nije bilo značajne razlike u odnosu na pol, stepen obrazovanja, zaposlenost, mesto stanovanja i bračni status ispitanika.
Zaključak: Svaka druga osoba u ovom istraživanju imala je neki vid anksioznosti. Neophodna su dalja istraživanja u ovoj oblasti i predlaganje adekvatnih preventivnih programa.

Ključne riječi: Kovid-19, pandemija, anksioznost, faktori rizika

Uvod

Svetska zdravstvena organizacija (SZO) je proglasila pandemiju koronavirusne bolesti 2019 (Kovid-19) 11. marta 2020. godine. Širom sveta je ograničeno kretanje stanovništva, uvedeno je obavezno nošenje zaštitnih maski, fizičko distanciranje od najmanje dva metra, obustavljena je nastava u školama i na fakultetima i limitiran je rad privrednih subjekata (1-3).

Fizičko distanciranje, ekonomski problemi, promene u obrazovnom sistemu, prestanak uobičajenog svakodnevnog funkcionisanja, pristup brojnim *web* stranicama sa ogromnim, ali ne uvek pouzdanim informacijama, strah od nedostatka zdravstvenih resursa i smrti negativno su uticali na psihološku dobrobit i mentalno zdravlje stanovništva. Zabeležen je porast porodičnog nasilja, zloupotrebe psihoaktivnih supstanci, depresije i anksioznosti (4-8).

Osim psiholoških osobina (načina suočavanja i nošenja sa stresom, uverenja o prirodi stresora, slike o sebi, optimizma ili pesimizma) i nivoa socijalne podrške u razvoju psihijatrijskih poremećaja, važnu ulogu imaju sociodemografski faktori ličnosti (4-8).

ORIGINAL ARTICLE

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THE INFLUENCE OF SOCIODEMOGRAPHIC FACTORS ON THE DEVELOPMENT OF ANXIETY DURING THE COVID-19 PANDEMIC

Marijana Jandric-Kocic[1], Snezana Knezevic[2]

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[2] Health Care Center Kraljevo, Serbia

SUMMARY

Introduction/Aim: The COVID-19 pandemic poses a serious threat to global mental health. Fear of death, social distancing, economic problems, changes in the education system, and cessation of normal daily functioning induce psychiatric disorders, primarily anxiety. The research aimed to determine the influence of sociodemographic factors on the development of anxiety during the COVID-19 pandemic.
Methods: The cross-sectional study, over two months, included 200 persons without symptoms of COVID-19, 60.0% of women and 40.0% of men, with an average age of 51.0 ± 9.2 years. One hundred people reported to the family medicine clinic of the Health Care Center Krupa on the River Una for examinations or administrative reasons, and another hundred were accompanied by them. In addition to the general questionnaire, the Generalized Anxiety Disorder-7 (GAD-7) Self-Assessment Symptom Intensity Questionnaire was used. The χ^2- test and Fisher's test were used for the statistical analysis.
Discussion: 105 (52.5%) participants included in the study had symptoms of anxiety (35.0% mild symptoms of anxiety, 15.5% moderate symptoms of anxiety, 2.0% severe symptoms of anxiety). Anxiety was slightly more common in men (56.2%) than in women (50.0%), as well as in the oldest (60-79 years) (92.6%) than in younger participants (12.5% for age 20-39 and 44.7% for ages 40-59). The frequency of anxiety was lower in persons with higher (33.3%) than lower education (44.5% of participants with primary and 46.6% of participants with high school). A slightly higher frequency of anxiety was among the employed (69.5%) than among the unemployed (43.0%), as well as among persons living in urban (80.8%) in comparison to those living in rural (42.6%) areas. Anxiety problems were experienced by 51.3% of participants living in marriage or extramarital union and 56.8% of participants who were not married or in an extramarital union. People with anxiety were significantly more likely to be aged 60-79 than people without anxiety. There were no significant differences between these groups in terms of gender, level of education, employment, place of residence and marital status of the participants.
Conclusion: Every other person in this study had some form of anxiety. Further research in this area and proposing adequate prevention programs are needed.

Key words: COVID-19, pandemic, anxiety, risk factors

Introduction

The World Health Organization (WHO) declared the pandemic of coronavirus disease 2019 (Covid-19) on 11 March, 2020. There have been movement restrictions around the world. The use of face masks is required now, as well as physical distancing of at least two meters. Schools and universities have been closed down and the work of business entities has been limited (1-3).

Physical distancing, economic problems, changes in the education system, the cessation of usual everyday activities, the access to numerous web pages with a lot of information, which is not always reliable, the fear of death and the lack of medical resources have had a negative influence on the psychological well-being and mental health of the population. The increase in family violence, the abuse of psychoactive substances, depression and anxiety have been recorded (4-8).

In addition to psychological traits (confronting and dealing with stress, opinion about the nature of stressors, an image of oneself, optimism, pessimism) and the level of social support in developing psychiatric disorders,

Identifikovanje vulnerabilnih grupa i obezbeđivanje psihosocijalne podrške kroz službe za socijalno i mentalno zdravlje predstavljaju važan deo zdravstvene zaštite u toku pandemije Kovid-19. Psihosocijalna procena i praćenje treba da obuhvate pitanja o stresorima povezanim sa Kovid-19 (izloženost izvorima zaraze, zaraženim članovima porodice, gubitak voljenih i socijalno distanciranje), sekundarne posledice (ekonomski gubitak), psihosocijalne efekte (depresija, anksioznost, povećana upotreba psihoaktivnih supstanci i porodično nasilje) i pokazatelje ranjivosti (postojeća psihološka stanja) (6-8).

Ova studija preseka imala je za cilj da utvrditi uticaj sociodemografskih faktora na razvoj anksioznosti u toku pandemije Kovid-19.

Metode

Studijom preseka bilo je obuhvaćeno 200 osoba bez simptoma Kovid-19 i to 100 osoba koje su se javile u ambulantu porodične medicine Doma zdravlja Krupa na Uni zbog pregleda ili administrativnih razloga, kao i po jedna osoba koja je bila u njihovoj pratnji. Istraživanje je trajalo od 15.03.2020. do 15.05.2020. godine. Kriterijumi za uključivanje ispitanika u istraživanje su bili: uzrast između 20 i 79 godina; a za isključivanje: maligne bolesti, psihijatrijska oboljenja i uznapredovale hronične bolesti (dijabetes, hronična bubrežna insuficijencija, insuficijencija jetre, dekompenzacija srca).

Opštim upitnikom prikupljeni su sociodemografski podaci (pol, uzrast, stepen obrazovanja, zaposlenost, mesto stanovanja i bračni status). Upitnik za samoprocenu intenziteta simptoma generalizovanog anksioznog poremećaja (engl. *Generalized Anxiety Disorder-7*, GAD-7) se koristi u proceni prisustva i intenziteta generalizovanog anksioznog poremećaja. Sastoji se od sedam pitanja koja mere težinu simptoma generalizovanog anksioznog poremećaja u toku poslednje dve nedelje (ocena od 0 do 3). Ukupan skor 0-4 odgovara osobama bez simptoma anksioznosti, 5-9 osobama sa blagim, 10-14 umerenim i ≥15 ozbiljno izdraženim simptomima anksioznosti (9-11).

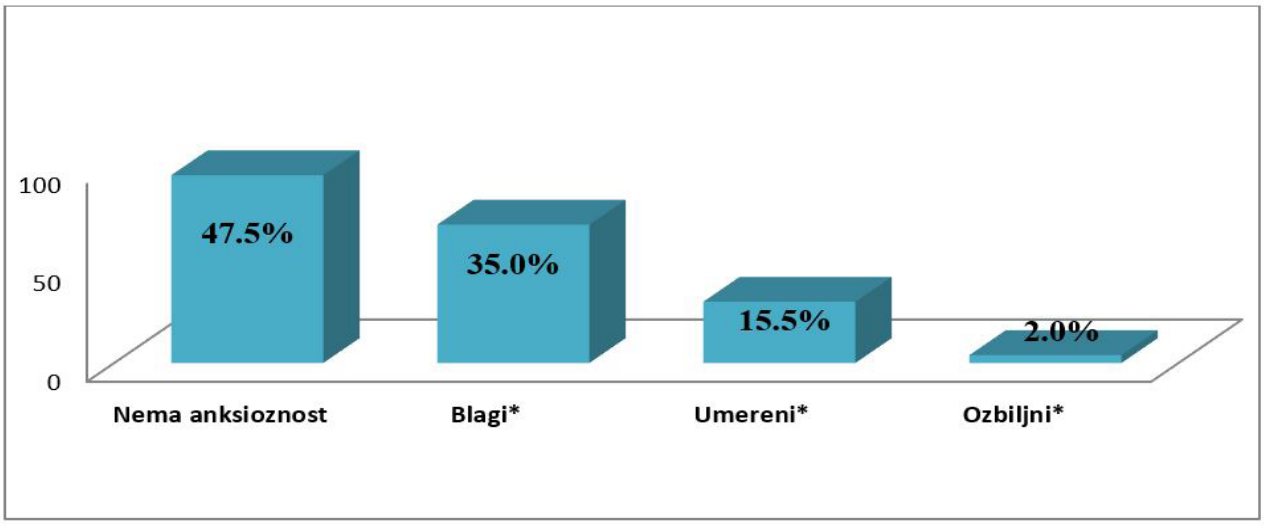
U detekciji generalizovanog anksioznog poremećaja upitnik ima visoku senzitivnost (89%) i specifičnost (82%) i prihvatljivu unutrašnju konzistentnost (*Cronbach alfa* = 0,75). Pilot validacija srpske verzije GAD-7 upitnika sprovedena je, 2019. godine, od strane Nikole Rokvića (12).

U statističkoj analizi podataka korišćeni su χ^2-test i *Fisher*-ov test.

Rezultati

Istraživanje je obuhvatilo 200 ispitanika. Prosečna starost ispitanika bila je 51,0 ± 9,2 godina. Simptome anksioznosti imalo je 52,5% (105) ispitanika i to 35,0% blage, 15,5% umerene i 2,0% ozbiljne (grafikon 1).

Anksioznost je utvrđena kod 56,2% muškaraca i 50,0% žena, kao i kod 12,5% ispitanika 20-39 godina, 44,7% ispitanika 40-59 godina



*simptomi anksioznosti

Grafikon 1. Distribucija ispitanika prema intenzitetu simptoma anksioznosti na osnovu Upitnika generalizovanog anksioznog poremećaja (GAD-7)

sociodemographic personality factors have an important role (4-8). Identifying vulnerable groups and providing psychosocial support through services for social and mental health present an important part of health protection during the Covid-19 pandemic. Psychosocial estimates and monitoring should include the questions about stressors connected with Covid-19 (exposure to the source of infection, infected family members, loss of the loved ones, and social distancing), secondary consequences (economic loss), psychosocial effects (depression, anxiety, the increased use of psychoactive substances and family violence) and indicators of vulnerability (existing psychological conditions) (6-8).

The aim of this cross-sectional study was to determine the influence of sociodemographic factors in the development of anxiety during the Covid-19 pandemic.

Methods

200 people without symptoms of Covid-19 were included in this study, that is, 100 people who reported to the family medicine clinic of the Health Care Center "Krupa on the River Una" for the examination or due to administrative reasons, and who were accompanied by 100 people. The research lasted from 15 March 2020 to 15 May 2020. The inclusion criterion was age between 20 and 79, while the exclusion criteria were the following: malign diseases, psychiatric diseases and severe chronic diseases (diabetes, chronic renal insufficiency, liver insufficiency, cardiac decompensation).

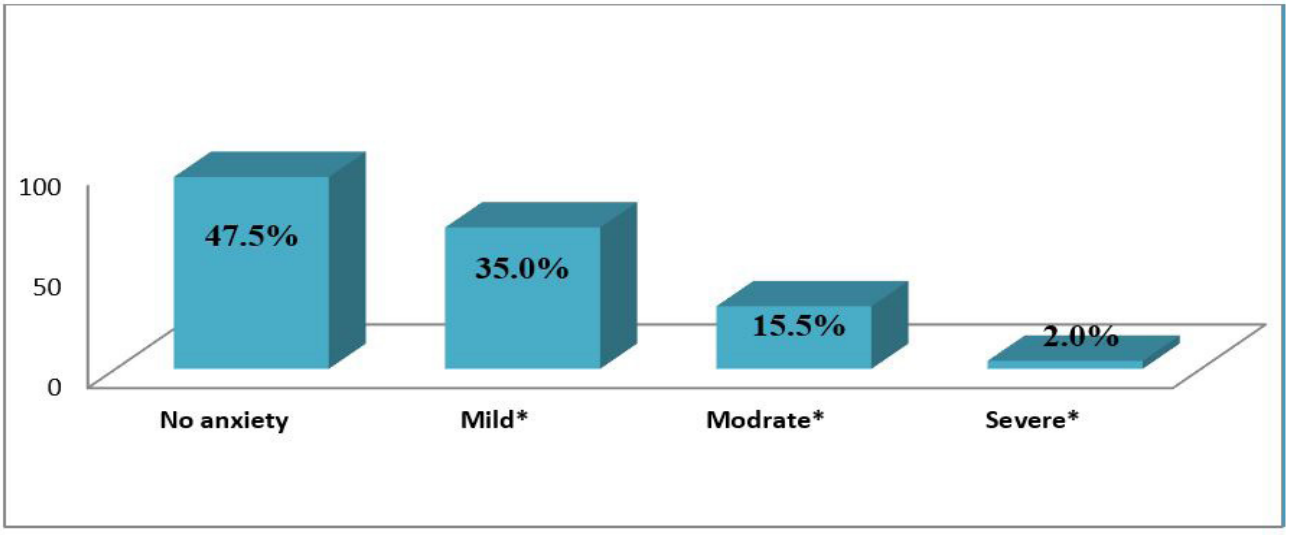
Sociodemographic data were collected with the help of a general questionnaire (gender, age, level of education, employment, place of living and marital status). The Generalized Anxiety Disorder 7 (GAD-7) self-assessment symptom itensity questionnaire was used to assess the presence and intensity of the generalized anxiety disorder. It consists of seven questions, which measure the severity of symptoms of the generalized anxiety disorder during the last two weeks (mark 0-3). The total score 0-4 relates to people without symptoms of anxiety, 5-9 to people with mild symptoms, 10-14 to moderate and >15 to severe symptoms of anxiety (9-11).

The questionnaire has high sensitivity (89%) and specificity (82%) and acceptable inner consistency (Cronbach alpha = 0.75) in detecting the generalized anxiety disorder. The pilot validation of the Serbian version of the GAD-7 Questionnaire was conducted in 2019 by Nikola Rokvic (12).

The χ^2 test and Fischer's test were used for the statistical analysis of data.

Results

The research included 200 participants. The mean age of the participants was 51.0 + 9.2 years. 52.5% (105) participants had the symptoms of anxiety, 35.0% of them had mild symptoms, 15.5% had moderate and 2.0% had severe symptoms (Graph 1).



*symptoms of anxiety

Graph 1. Distribution of participants according to the intensity of anxiety symptoms based on the Generalized Anxiety Disorder (GAD-7) questionnaire

Tabela 1. Prevalencija simptoma anksioznosti prema demografskim karakteristikama ispitanika

Karakteristike/*Characteristics*		**Prevalencija simptoma anksioznosti (%)**/ *Prevalence of anxiety symptoms (%)*
Pol/***Gender***	Muškarci/*Men* (N=80)	56.2
	Žene/*Women* (N=120)	50.0
Uzrast (godine)/ ***Age (years)***	20-39 (N=32)	12.5
	40-59 (N=114)	44.7
	60-79 (N=54)	92.6
Stepen obrazovanja/ ***Level of education***	Osnovna škola/ *Primary school* (N=6)	44.5
	Srednja škola/ *High school* (N=182)	46.6
	Fakultet/*College* (N=12)	33.3
Zaposlen/ ***Employed***	Da/ *Yes* (N=72)	69.5
	Ne/ *No* (N=128)	43.0
Mesto stanovanja/ ***Place of Residence***	Selo/*Village* (N=52)	42.6
	Grad/*City* (N=148)	80.8
Bračni status/ ***Marital status***	U braku ili vanbračnoj zajednici/ *Married or extramarital union* (N=156)	51.3
	Nisu u braku ili vanbračnoj zajednici/N*ot married or extramarital union* (N=44)	56.8

i 92,6% ispitanika 60-79 godina (tabela 1). Anksiozne tegobe je imalo 44,5% ispitanika sa osnovnom školom, 46,6% ispitanika sa srednjom školom i 33,3% ispitanika sa fakultetom. Takođe, anksioznost je utvrđena kod 69,5% zaposlenih i 43,0% nezaposlenih, kao i kod 80,8% osoba koje žive u urbanoj i 42,6% osoba koje žive u ruralnoj sredini. Svaka druga osoba u braku ili vanbračnoj zajednici (51,3%), kao i osobe koje nisu bile u braku ili vanbračnoj zajednici (56,8%), imala je anksioznost.

Osobe sa anksioznošću su značajno češće bile uzrasta 60-79 godina, nego osobe bez anksioznosti (tabela 2). Između ispitanika sa i bez anksioznosti nije postojala značajna razlika u odnosu na pol, stepen obrazovanja, zaposlenost, mesto stanovanja i bračni status.

Osobe sa blagim simptomima anksioznosti (tabela 3), kao i osobe sa umerenim i ozbiljnim simptomima (tabela 4), su značajno češće bile uzrasta 60-79 godina, nego osobe bez anksioznosti. U odnosu na druge sociodemogrfaske karakteristike nisu postojale značajne razlike između ispitivanih grupa.

Diskusija

Pandemija Kovid-19 predstavlja ozbiljnu pretnju globalnom mentalnom zdravlju. Neizvesna prognoza, preteći nedostatak resursa, izricanje do sada nepoznatih javno-zdravstvenih mera indukovale su psihijatrijske poremećaje, u prvom redu anksioznost (8,13,14). Brojna istraživanja ukazuju na značajan porast anksioznosti izazvan SARS-CoV-2 virusom (16-22).

Naše istraživanje je utvrdilo postojanje anksioznosti kod 52,5% ispitanika (35,0% blaga, 15,5% umerena i 2,0% ozbiljna anksioznost). Istraživanje (4.827 ispitanika) sprovedeno u Kini (rana faza epidemije Kovid-19) utrdilo je da je prevalencija umerene i teške anksioznosti 22,6 – 28,8% (15,16). U velikoj studiji kineskih autora (7.236 učesnika) u martu 2020. godine prevalencija anksioznosti iznosila je 35,1 % (10). Istraživanje sprovedeno u Americi verifikovalo je prisustvo anksioznosti kod 36,5% učesnika (18). Studija sprovedena u Indiji utvrdila je postojanje anksioznosti kod više od 30,0% učesnika u istraživanju (17). U istraživanju iranskih autora prevalencija anksioznosti iznosila je 50,9% (10,5% blaga,

Table 1. Prevalence of anxiety symptoms according to demographic characteristics of participants

Karakteristike/ ***Characteristics***		**Prevalencija simptoma anksioznosti (%)/** ***Prevalence of anxiety symptoms (%)***
Pol/*Gender*	Muškarci/*Men* (N=80)	56.2
	Žene/*Women* (N=120)	50.0
Uzrast (godine)/ ***Age (years)***	20-39 (N=32)	12.5
	40-59 (N=114)	44.7
	60-79 (N=54)	92.6
Stepen obrazovanja/ ***Level of education***	Osnovna škola/ *Primary school* (N=6)	44.5
	Srednja škola/ *High school* (N=182)	46.6
	Fakultet/*College* (N=12)	33.3
Zaposlen/ ***Employed***	Da/ *Yes* (N=72)	69.5
	Ne/ *No* (N=128)	43.0
Mesto stanovanja/ ***Place of residence***	Selo/*Village* (N=52)	42.6
	Grad/*City* (N=148)	80.8
Bračni status/ ***Marital status***	U braku ili vanbračnoj zajednici/ *Married or extramarital union* (N=156)	51.3
	Nisu u braku ili vanbračnoj zajednici/N*ot married or extramarital union* (N=44)	56.8

Anxiety was confirmed in 56.2% of men and 50.0% of women, as well as in 12.5% of participants aged 20-39, in 44.7% of participants aged 40-59, and in 92.6% of participants aged 60-79 (Table 1). Anxiety was experienced by 44.5% of participants with primary school, 46.6% of participants with high school and 33.3% of them with faculty education. Also, anxiety was confirmed in 69.5% of employed people and 43.0% of unemployed, as well as in 80.8% of people living in urban areas and 42.6% of those living in rural areas. Every other person (51.3%), who was married or lived in an extramarital union, experienced anxiety, as well as people who were not married or did not live in extramarital union (56.8%).

People with anxiety were significantly more often aged 60-79 in comparison to people without anxiety (Table 2). There was no significant statistical difference between people with and without anxiety regarding gender, level of education, employment, place of living and marital status.

People with mild forms of anxiety (Table 3), as well as people with moderate and severe symptoms (Table 4) were significantly more often in the age group 60-79 than people without anxiety symptoms. There was no significant statistical difference between the examined groups regarding other sociodemographic factors.

Discussion

The Covid-19 pandemic poses a serious threat to global mental health. Uncertain prognoses, threatening lack of resources, passing the unprecedented public health measures induced psychiatric disorders, first of all, anxiety (8,13,14). Numerous research studies have pointed to the significant increase of anxiety caused by the virus SARS-CoV-2 (16-22).

The existence of anxiety was confirmed in our research in 52.5% of participants (35.0% mild, 15.5% moderate, and 2.0% severe anxiety). One research study (4827 participants, which was conducted in China (in the early phase of Covid-19 epidemic), confirmed the prevalence of moderate and severe anxiety 22.6% - 28.8% (15,16). In a big study of Chinese authors (7236 participants), the prevalence of anxiety amounted to 35.1% in March, 2020

Tabela 2. Distribucija ispitanika sa i bez anksioznosti prema njihovim demografskim karakteristikama

Karakteristike/ ***Characteristics***		**Bez anksioznosti/** ***No anxiety*** **(N=95) Broj (%)/No(%)**	**Sa anksioznošću/** ***With anxiety*** **(N=105) Broj (%)/No(%)**	**p vrednost*/** ***p value****
Pol/*Gender*	Muškarci/*Men*	35 (36.8)	45 (42.9)	
	Žene/*Women*	60 (63.2)	60 (57.1)	p > 0.05
Uzrast (godine)/ ***Age (years)***	20-39	28 (29.5)	4 (3.8)	
	40-59	63 (66.3)	51 (48.6)	
	60-79	4 (4.2)	50 (47.6)	p < 0.05
Stepen obrazovanja/ ***Level of education***	Osnovna škola/ *Primary school*	0 (0.0)	6 (5.7)	
	Srednja škola/ *High school*	87 (91.6)	95 (90.5)	
	Fakultet/*College*	8 (8.4)	4 (3.8)	p > 0.05
Zaposlen/ ***Employed***	Da/ *Yes*	22 (23.2)	50 (47.6)	
	Ne/ *No*	73 (76.8)	55 (52.4)	p > 0.05
Mesto stanovanja/ ***Place of Residence***	Selo/*Village*	85 (89.5)	63 (60.0)	
	Grad/*City*	10 (10.5)	42 (40.0)	p > 0.05
Bračni status/ ***Marital status***	U braku ili vanbračnoj zajednici/ *Married or extramarital union*	76 (80.0)	80 (76.2)	
	Nisu u braku ili vanbračnoj zajednici/N*ot married or extramarital union*	19 (20.0)	25 (23.8)	p > 0.05

Prema χ^2 ili *Fisher*-ovom testu / **According to χ^2 or Fisher test*

21,3% umerena, 9,3% ozbiljna i 9,8% veoma ozbiljna anskioznost) (19). Istraživanje medicinskih i nemedicinskih zdravstvenih radnika u Singapuru utrvdilo je prevalenciju anksioznosti između 14,5 i 20,7% (20). Istraživanje sprovedeno među 1.830 medicinskih radnika zaposlenih u Kovid bolnicama verifikovalo je postojanje anksioznosti kod 44,6% učesnika (21). U istraživanju sprovedenom među pacijentima hospitalizovanim zbog Kovida-19 prevalencija anksioznosti iznosila je 34,7% (22).

U našoj studiji, prevalencija anksioznosti je bila najveća kod ispitanika uzrasta 60-79 godina. Ispitanici sa anksioznošću značajno su češće bili uzrasta 60-79 godina, nego ispitanici bez anksioznosti. Takođe, ispitanici sa blagom, kao i sa srednje teškom ili teškom, anksioznošću su značajno češće bili uzrasta 60-79 godina, nego ispitanici bez anksioznosti. Istraživanja autora iz Velike Britanije došla su do sličnih rezultata. Prema istim, starija životna dob je povezana sa prisustvom multimorbiditeta i značajnom socijalom izolacijom (23). Istraživanja sprovedena u Sjedinjenim Američkim Državama i Kini utvrdila su značajno češće prisustvo anksioznosti kod osoba mlađe životne dobi (< 35 godina). Mlade osobe su provele više vremena i to ≥ 3 sata razmišljajući o Kovidu-19, što je značajno povezano sa razvojem anksioznosti (10,17).

Između ispitanika sa anksioznošću, kao i kada su u pitanju ispitanici sa blagom i srednje teškom ili teškom anksioznošću, i ispitanika bez anksioznosti nije bilo značajne razlike u odnosu na pol, stepen obrazovanja, zaposlenost, mesto stanovanja i bračni status. Međutim, prevalencija anksioznosti je bila nešto veća za muškarce nego žene. Pol nije predstavljao značajan prediktor razvoja anskioznosti u Nacionalnoj internetskoj anketi o mentalnom zdravlju sprovedenoj u Kini (10). Druge studije kineskih autora utvrdile su značajno višu anksioznost kod osoba ženskog pola. Osim značajno većeg rizika od razvoja anksioznosti (kao posledice rodnih razlika u

Table 2. Distribution of participants with and without anxiety according to their demographic characteristics

Karakteristike/ ***Characteristics***		**Bez anksioznosti/** ***No anxiety*** **(N=95) Broj (%)/No(%)**	**Sa anksioznošću/** ***With anxiety*** **(N=105) Broj (%)/No(%)**	**p vrednost*/** ***p value****
Pol/*Gender*	Muškarci/*Men*	35 (36.8)	45 (42.9)	
	Žene/*Women*	60 (63.2)	60 (57.1)	p > 0.05
Uzrast (godine)/ ***Age (years)***	20-39	28 (29.5)	4 (3.8)	
	40-59	63 (66.3)	51 (48.6)	
	60-79	4 (4.2)	50 (47.6)	p < 0.05
Stepen obrazovanja/ ***Level of education***	Osnovna škola/ *Primary school*	0 (0.0)	6 (5.7)	
	Srednja škola/ *High school*	87 (91.6)	95 (90.5)	
	Fakultet/*College*	8 (8.4)	4 (3.8)	p > 0.05
Zaposlen/ ***Employed***	Da/ *Yes*	22 (23.2)	50 (47.6)	
	Ne/ *No*	73 (76.8)	55 (52.4)	p > 0.05
Mesto stanovanja/ ***Place of Residence***	Selo/*Village*	85 (89.5)	63 (60.0)	
	Grad/*City*	10 (10.5)	42 (40.0)	p > 0.05
Bračni status/ ***Marital status***	U braku ili vanbračnoj zajednici/ *Married or extramarital union*	76 (80.0)	80 (76.2)	
	Nisu u braku ili vanbračnoj zajednici/N*ot married or extramarital union*	19 (20.0)	25 (23.8)	p > 0.05

*Prema χ^2 ili *Fisher*-ovom testu / **According to χ^2 or Fisher test*

(10). A research conducted in America verified the presence of anxiety in more than 36.5% of participants (18). A study conducted in India determined the existence of anxiety in more than 30.0% of research participants (17). In a research study of Iranian authors, the prevalence of anxiety amounted to 50.9% (10.5% mild, 21.3% moderate, 9.3% severe and 9.8% very severe) (19). A research among medical and non-medical health care workers in Singapore determined the prevalence f anxiety between 14.5 – 20.7% (20). A research among health care workers employed at the Covid hospitals (1830 participants) verified the presence of anxiety in 44.6% of cases (21). In a research conducted among the patients hospitalized due to Covid-19, the prevalence of anxiety amounted to 34.7% (22).

In our study, the prevalence of anxiety was highest among the participants aged 60-79. Participants with anxiety were significantly more often in the age group 60-79 in comparison to participants without anxiety. Also, participants with the mild, as well as with the moderately severe and severe anxiety were significantly more often in the age group 60-79 than participants without anxiety. Studies of the authors from Great Britain came to similar results. According to them, older age is associated with the presence of multi-morbidities and significant social isolation (23). Research studies conducted in the USA and China confirmed a significantly more frequent presence of anxiety in younger people (<35 years). Young people spent more time, that is > 3 hours, thinking about Covid-19, which is associated with the development of anxiety (10,17).

There was no significant difference between participants with anxiety, with mild and moderately severe or severe anxiety and participants without anxiety regarding gender, level of education, employment, place of living and marital status. However, the prevalence of

Tabela 3. Distribucija ispitanika sa blagim simptomima anksioznosti i bez anksioznosti prema njihovim demografskim karakteristikama

Karakteristike/ ***Characteristics***		**Bez anksioznosti/** ***No anxiety*** **(N=95) Broj (%)/No(%)**	**Sa anksioznošću/** ***With anxiety*** **(N=105) Broj (%)/No(%)**	**p vrednost*/** ***p value****
Pol/*Gender*	Muškarci/*Men*	35 (36.8)	30 (42.9)	
	Žene/*Women*	60 (63.2)	40 (57.1)	p > 0.05
Uzrast (godine)/ ***Age (years)***	20-39	28 (29.5)	3 (4.3)	
	40-59	63 (66.3)	47 (67.1)	
	60-79	4 (4.2)	20 (28.6)	p < 0.05
Stepen obrazovanja/ ***Level of education***	Osnovna škola/ *Primary school*	0 (0.0)	2 (2.9)	
	Srednja škola/ *High school*	87 (91.6)	66 (73.3)	
	Fakultet/*College*	8 (8.4)	2 (2.9)	p > 0.05
Zaposlen/ ***Employed***	Da/ *Yes*	22 (23.2)	28 (40.0)	
	Ne/ *No*	73 (76.8)	42 (60.0)	p > 0.05
Mesto stanovanja/ ***Place of Residence***	Selo/*Village*	85 (89.5)	48 (68.6)	
	Grad/*City*	10 (10.5)	22 (31.4)	p > 0.05
Bračni status/ ***Marital status***	U braku ili vanbračnoj zajednici/ *Married or extramarital union*	76 (80.0)	60 (85.7)	
	Nisu u braku ili vanbračnoj zajednici/N*ot married or extramarital union*	19 (20.0)	10 (14.2)	p > 0.05

*Prema χ^2 ili *Fisher*-ovom testu / **According to χ^2 or Fisher test*

ulogama u savremenom društvu) kod žena je utvrđen značajno veći nivo stresa tokom Kovid-19 pandemije (14,16).

U našoj studiji, suprotno drugim istraživanjima, prevalencija anksioznosti je bila skoro duplo veća u gradu nego na selu. Istraživanje sprovedeno među kineskim studentima utvrdilo je značajno manju anksioznost kod osoba sa mestom prebivališta u gradu. Prema istom, urbana sredina je povezana sa značajno većim kulturnim, obrazovnim i ekonomskim resursima. Gradovi imaju bolje sanitarne uslove (smanjene šanse za preživljavanje virusa), veće obrazovne resurse (kvalitetnija edukacija stanovništva o merama prevencije) i razvijeniju ekonomiju (izvor materijalne sigurnosti) (15).

U našoj studiji, kao i u nekoliko studija kineskih autora uočeno je značajno viša anksioznost kod ispitanika sa nižim stepenom obrazovanja. Prema istim značajan broj osoba nižeg obrazovanja ima niska primanja ili su nezaposleni. Istraživanje američkih autora utvrdilo je značajno vulnerabilnije mentalno zdravlje kod osoba sa godišnjim primanjima manjim od 40.000 američkih dolara (15,24). S druge strane, anketiranje sprovedeno putem interneta u Americi je utvrdilo značajno veću anksioznost kod fakultetski obrazovanih osoba. Povećan broj anksioznih osoba među osobama sa visokom stručnom spremom, može se objasniti činjenicom da fakultetsko obrazovanje ne garantuje odgovarajuću platu ili sigurnost posla tokom pandemije Kovid-19 (17).

Naše istraživanje ukazuje da je prevalencija anksioznosti nešto veća kod ispitanika koji su zaposleni nego nezaposleni. U zemljama koje nemaju pokrivenost zdravstvenog osiguranja na nacionalnom nivou i prihvatljive ciljeve zdravstvenih usluga nezaposlena lica mogu imati finansijskih problema u ostvarivanju neophodnih zdravstvenih usluga. Egzistencijalni problemi, neadekvatni uslovi stanovanja i slabiji pristup informacionim tehnologijama mogu doprineti nastanku anksioznosti osoba

Table 3. Distribution of participants with mild symptoms of anxiety and without anxiety according to their demographic characteristics

Karakteristike/ ***Characteristics***		**Bez anksioznosti/** ***No anxiety*** **(N=95) Broj (%)/No(%)**	**Sa anksioznošću/** ***With anxiety*** **(N=105) Broj (%)/No(%)**	**p vrednost*/** ***p value****
Pol/*Gender*	Muškarci/*Men*	35 (36.8)	30 (42.9)	
	Žene/*Women*	60 (63.2)	40 (57.1)	p > 0.05
Uzrast (godine)/ ***Age (years)***	20-39	28 (29.5)	3 (4.3)	
	40-59	63 (66.3)	47 (67.1)	
	60-79	4 (4.2)	20 (28.6)	p < 0.05
Stepen obrazovanja/ ***Level of education***	Osnovna škola/ *Primary school*	0 (0.0)	2 (2.9)	
	Srednja škola/ *High school*	87 (91.6)	66 (73.3)	
	Fakultet/*College*	8 (8.4)	2 (2.9)	p > 0.05
Zaposlen/ ***Employed***	Da/ *Yes*	22 (23.2)	28 (40.0)	
	Ne/ *No*	73 (76.8)	42 (60.0)	p > 0.05
Mesto stanovanja/ ***Place of Residence***	Selo/*Village*	85 (89.5)	48 (68.6)	
	Grad/*City*	10 (10.5)	22 (31.4)	p > 0.05
Bračni status/ ***Marital status***	U braku ili vanbračnoj zajednici/ *Married or extramarital union*	76 (80.0)	60 (85.7)	
	Nisu u braku ili vanbračnoj zajednici/N*ot married or extramarital union*	19 (20.0)	10 (14.2)	p > 0.05

*Prema χ^2 ili *Fisher*-ovom testu / **According to χ^2 or Fisher test*

anxiety was somewhat higher in men than in women. Gender was not a significant predictor of anxiety development in the National Internet Survey of mental health conducted in China (10). Other studies of Chinese authors found significantly higher anxiety in female participants. In addition to a significantly higher risk of developing anxiety (as a consequence of gender differences regarding the roles in the contemporary society), there was a significantly higher level of stress in women during the Covid-19 pandemic (14,16).

In our study, contrary to other research studies, the prevalence of anxiety was almost two times higher in urban than in rural areas. A research, which was conducted among Chinese students, found significantly lower anxiety in people living in cities. According to this research, urban areas are associated with significantly greater cultural, educational and economic resources. Cities have better sanitary conditions (chances are smaller that the virus can survive), greater educational resources (better quality education about preventive measures) and a more developed economy (source of financial security) (15).

In our study, as well as in several studies of Chinese authors, it was noticed that anxiety was significantly more severe in participants with lower level of education. According to them, a significant number of people with lower level of education have lower wages or they are unemployed. A study of American authors found significantly more vulnerable mental health in people with annual salary lower than 40.000 American dollars (15,24). On the other hand, a survey conducted on the Internet in America found a significantly higher anxiety in people who finished college. A greater number of people with academic degrees, as well as the fact that higher education does not guarantee the appropriate salary or job security, are stated as possible explanations (17).

Tabela 4. Distribucija ispitanika sa umerenim ili ozbiljnim simptomima anksioznosti i bez aksioznosti prema njihovim demografskim karakteristikama

Karakteristike/ ***Characteristics***		**Bez anksioznosti/** ***No anxiety*** **(N=95) Broj (%)/No(%)**	**Sa anksioznošću/** ***With anxiety*** **(N=105) Broj (%)/No(%)**	**p vrednost*/** ***p value****
Pol/*Gender*	Muškarci/*Men*	35 (36.8)	15 (42.9)	
	Žene/*Women*	60 (63.2)	20 (57.1)	p > 0.05
Uzrast (godine)/ ***Age (years)***	20-39	28 (29.5)	1 (2.9)	
	40-59	63 (66.3)	4 (11.4)	
	60-79	4 (4.2)	30 (85.7)	p < 0.05
Stepen obrazovanja/ ***Level of education***	Osnovna škola/ *Primary school*	0 (0.0)	4 (11.4)	
	Srednja škola/ *High school*	87 (91.6)	29 (82.9)	
	Fakultet/*College*	8 (8.4)	2 (5.7)	p > 0.05
Zaposlen/ ***Employed***	Da/ *Yes*	22 (23.2)	22 (62.9)	
	Ne/ *No*	73 (76.8)	13 (37.1)	p > 0.05
Mesto stanovanja/ ***Place of Residence***	Selo/*Village*	85 (89.5)	15 (42.9)	
	Grad/*City*	10 (10.5)	20 (57.1)	p > 0.05
Bračni status/ ***Marital status***	U braku ili vanbračnoj zajednici/ *Married or extramarital union*	76 (80.0)	20 (57.1)	
	Nisu u braku ili vanbračnoj zajednici/N*ot married or extramarital union*	19 (20.0)	15 (42.9)	p > 0.05

*Prema χ^2 ili *Fisher*-ovom testu / **According to χ^2 or Fisher test*

bez stalnog zaposlenja tokom pandemije nove koronavirusne bolesti (16). Međutim, anksioznost zaposlenih je najverovatnije usled straha od gubitka posla.

Naši rezultati ukazuju da život u braku ili bračnoj zajednici ne doprinosi nižoj prevalenciji simptoma anksioznosti u odnosu na one koji ne žive u braku ili bračnoj zajednici. Suprotno našim rezultatima, istraživanja sprovedena u Kini utvrdila su značajno manju anksioznost osoba koje žive u braku ili vanbračnoj zajednici, jer zajednički život i roditeljstvo ostavljaju manje vremena za razmišljajanje o ovoj bolesti (10).

Naše istraživanje je sprovedeno u ranoj fazi pandemije Kovid-19 i kao takvo dalo je svoj doprinos u pravovremenoj identifikaciji vulnerabilnih kategorija prema kojima bi trebalo usmeriti psihosocijalne intervencije u cilju pre-vencije anksioznosti. Limitirano vreme trajanja istraživanja i mali broj ispitanika može predstavljati ograničenje istraživanja.

Zaključak

Istraživanje je pokazalo da je anksioznost prisutna kod nešto više od polovine ispitanika tokom pandemije Kovid-19. Najveći broj ispitanika imao je blagu anksioznost, dok je ozbiljna anksioznost bila retka. Osobe sa anksioznošću su značajno češće bile starijeg uzrasta (60-79 godina) nego osobe bez anksioznosti. Između ispitanika sa i bez anksioznosti nije bilo značajne razlike u odnosu na sve druge sociodemografske faktore. Neophodna su dalja istraživanja u ovoj oblasti i primena adekvatnih preventivnih programa.

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Table 4. Distribution of participants with moderate or severe symptoms of anxiety and without anxiety according to their demographic characteristics

Karakteristike/ ***Characteristics***		**Bez anksioznosti/** ***No anxiety*** **(N=95) Broj (%)/No(%)**	**Sa anksioznošću/** ***With anxiety*** **(N=105) Broj (%)/No(%)**	**p vrednost*/** ***p value****
Pol/*Gender*	Muškarci/*Men*	35 (36.8)	15 (42.9)	
	Žene/*Women*	60 (63.2)	20 (57.1)	p > 0.05
Uzrast (godine)/ ***Age (years)***	20-39	28 (29.5)	1 (2.9)	
	40-59	63 (66.3)	4 (11.4)	
	60-79	4 (4.2)	30 (85.7)	p < 0.05
Stepen obrazovanja/ ***Level of education***	Osnovna škola/ *Primary school*	0 (0.0)	4 (11.4)	
	Srednja škola/ *High school*	87 (91.6)	29 (82.9)	
	Fakultet/*College*	8 (8.4)	2 (5.7)	p > 0.05
Zaposlen/ ***Employed***	Da/ *Yes*	22 (23.2)	22 (62.9)	
	Ne/ *No*	73 (76.8)	13 (37.1)	p > 0.05
Mesto stanovanja/ ***Place of Residence***	Selo/*Village*	85 (89.5)	15 (42.9)	
	Grad/*City*	10 (10.5)	20 (57.1)	p > 0.05
Bračni status/ ***Marital status***	U braku ili vanbračnoj zajednici/ *Married or extramarital union*	76 (80.0)	20 (57.1)	
	Nisu u braku ili vanbračnoj zajednici/N*ot married or extramarital union*	19 (20.0)	15 (42.9)	p > 0.05

Prema χ^2 ili *Fisher*-ovom testu / **According to χ^2 or Fisher test*

Our study pointed out that the prevalence of anxiety was somewhat higher in participants who were employed than in unemployed ones. In countries, which do not have health insurance at the national level and acceptable goals of health services, unemployed people can have financial problems when getting necessary health services. Existential problems, inadequate conditions of living and poor access to information technologies can lead to the appearance of anxiety in people without permanent jobs during the pandemic of the novel coronavirus disease (16). However, anxiety among employed people most likely appears due to the fear of losing a job.

Our results show that marital life and life in extramarital unions do not contribute to the lower prevalence of anxiety symptoms in comparison to those who are not married. Contrary to our results, research studies conducted in China found significantly lower anxiety in people who were married or lived in extramarital unions, because living together and parenting leave less time for thinking about this disease (10).

Our research was conducted in the early phase of the Covid-19 pandemic, and therefore, it gave its contribution to the timely identification of vulnerable categories, according to which, psychosocial interventions should be directed in order to prevent anxiety. Limited time of the research and a smaller number of participants could represent the limits of this research.

Conclusion

The research showed that anxiety was present in more than half of the participants during the Covid-19 pandemic. The largest number of participants had mild anxiety, whereas severe anxiety was rare. People with anxiety were significantly more often in the older age group (60-79 years) than people without anxiety. There was no significant difference between participants with and

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without anxiety regarding gender and other sociodemographic factors. Further research in this field is necessary, as well as the application of appropriate prevention programs.

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DIJAGNOSTIČKI I TERAPIJSKI IZAZOVI U LEČENJU PACIJENATA TOKOM KOVID-19 EPIDEMIJE

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SAŽETAK

Uvod: Početak decembra 2019. godine se smatra periodom početka infekcije novim korona virusom (engl. S*evere Acute Respiratory Syndrome Coronavirus 2* - SARS-CoV-2), u Vuhanu, u Kini, koja je stručnoj javnosti poznatija kao Kovid-19 (engl. *Coronavirus Disease 2019* - COVID-19).
Prikaz slučaja: Pacijent muškog pola, starosti 42 godine, upućen je na pregled zbog sumnje na Kovid-19. Prema rečima pacijenta, simptomi su počeli četiri dana ranije pojavom mučnine i kašlja, a potom otežanog disanja i povišene temperature. Tokom pregleda, pacijent je bio bled, otežanog disanja, subfebrilan, tahikardičan i normotenzivan. Radiološkim pregledom verifikovan je težak oblik Kovid-19 pneumonije. Laboratorijski parametri ukazivali su na infektivni sindrom i srčanu slabost. Prvog dana hospitalizacije došlo je do pogoršanja opšteg stanja: pojavom aritmije, potom respiratornog pogoršanja. Obezbeđena je terapijska potpora prema Kovid-19 protokolu. Drugog dana hospitalizacije pacijent je intubiran, sprovedena je trodnevna mehanička ventilatorna potpora, vazopresorna potpora hemodinamike, dvojna stimulacija diureze, nakon koje dolazi do stabilizacije vitalnih parametra. Ponavljano *Polymerase Chain Reaction* (PCR) testiranje i testiranje iz bronho-alveolarnog lavata (BAL) bilo je negativno. Ponovljeni rendgenski snimak ukazao je na značajnu regresiju nalaza na plućima. Urađen je ultrazvučni pregled srca koji je ukazao na značajno redukovanu funkciju leve komore, u poređenju sa prethodnim pregledom. Konsultovan je kardiohirurg koji je potvrdio neophodnost operativnog lečenja valvularne mane na aortnoj i mitralnoj poziciji uz prethodnu sanaciju infekcije i kardiološku pripremu. Dva meseca nakon ove hospitalizacije, urađen je rekonstruktivni kardiohirurški zahvat.
Zaključak: Prikazom kliničkog toka bolesti, dijagnostičkog i terapijskog pristupa kod pacijenta sa kardiovaskularnim komorbiditetom hteli smo da ukažemo i na poteškoće u prepoznavanju kliničke slike i dijagnostikovanju Kovida-19.

Ključne reči: Kovid-19, pneumonija, mehanička ventilacija, prikaz slučaja

Uvod

Početak decembra 2019. godine se smatra periodom početka infekcije novim koronavirusom (engl. *Severe Acute Respiratory Syndrome Coronavirus 2* - SARS-CoV-2), u Vuhanu, u Kini, koja je stručnoj populaciji poznatija kao Kovid-19 (engl. *Coronavirus Disease 2019* - COVID-19) (1). Prema izveštaju Svetske zdravstvene organizacije (SZO) na dan 21.09.2020. od decembra 2019. godine do 21.09.2020. je u celom svetu, od Kovid-19 infekcije obolelo više od 31 milion ljudi, a preminulo 959.116 osoba, sa letalitetom od 3,1% (2). Prema dnevnom izveštaju Instituta za javno zdravlje Republike Srbije „Dr Milan Jovanović Batut", na dan 21.09.2020. godine, od početka epidemije u Republici Srbiji je od Kovid-19 obolelo 32.938 osoba, dok su 743 preminule od posledica infekcije, sa letalitetom od 2,3% (3).

Prikaz slučaja

Pacijent muškog pola, starosti 42 godine, upućen je na pregled na Kliniku za infektivne i tropske bolesti Kliničkog centra Srbije (KCS) zbog sumnje na Kovid-19. Prema rečima pacijenta, simptomi su počeli četiri dana ranije pojavom mučnine i kašlja, potom otežanog

CASE REPORT

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DIAGNOSTIC AND THERAPEUTIC CHALLENGES IN THE TREATMENT OF PATIENTS DURING THE COVID-19 EPIDEMIC

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SUMMARY

Introduction: The beginning of December 2019 is considered to be the beginning of infection with the new coronavirus (Severe Acute Respiratory Syndrome Coronavirus 2 - SARS-CoV-2), in Wuhan, China, better known among professionals as COVID-19 (Coronavirus Disease 2019 -COVID-19).
Case report: A 42-year-old male was referred for examination due to suspicion of COVID-19. According to the patient, the symptoms started four days ago with nausea and cough, heavy breathing, and fever. During the examination, the patient was pale, breathed with difficulty, subfebrile, tachycardic, normotensive. A severe form of COVID 19 pneumonia was verified by the radiological examination. Laboratory parameters indicated the infectious syndrome and heart failure. On the first day of hospitalization, the general condition worsened: with the appearance of arrhythmia, then respiratory deterioration. The therapeutic support was provided according to the COVID-19 protocol. On the second day of hospitalization, the patient was intubated, followed by three-day mechanical ventilatory support, vasopressor stimulation of hemodynamics, double stimulation of diuresis. All steps provided vital parameter stabilization. Repeated Polymerase Chain Reaction (PCR) testing and bronchoalveolar lavage (BAL) testing were negative. Repeated chest x-ray indicated the significant regression of findings. A heart ultrasound was performed, which indicated a significantly reduced function of the left ventricle in comparison to the previous examination. The patient had previous cardiac co-morbidities and the cardiac surgeon gave an opinion and confirmed the need for surgical correction of aortic and mitral valves disease with the previous treatment of the infection. Two months later, reconstructive cardiac surgery was performed.
Conclusion: By presenting the clinical course of the disease, diagnostic, and therapeutic approach in a patient with cardiovascular comorbidity, we wanted to point out the difficulties in recognizing the clinical picture and diagnosing COVID-19.

Key words: COVID-19, pneumonia, mechanic ventilatory support, case report

Introduction

The beginning of December 2019 is considered to be the beginning of the infection with the novel coronavirus (Severe Acute Respiratory Syndrome Coronavirus 2 - SARS-CoV-2,) in Wuhan, in China, which is known as COVID-19 among professionals (1). According to the report of the World Health Organization from December 2019 to September 21st, 2020 more than 31 million people were infected by the COVID-19, while 959.116 died, with the lethality of 3.1% (2). According to the daily report of the Public Health Institute of Serbia "Dr Milan Jovanovic Batut" on September 21st, 2020, 32,938 people got infected with COVID-19 in the Republic of Serbia from the beginning of the epidemic, while 743 died of the consequences of this infection, with the lethality of 2.4% (3).

A Case Report

A 42 year old male patient was referred to the Clinic for Infectious and Tropical Diseases of the Clinical Centre of Serbia due to the suspicion of Covid-19. According to him, symptoms started

disanja i povišene temperature. Za to vreme, koristio je simptomatsku terapiju. Pacijent negira prisustvo alergija na lekove i ranije operativne zahvate. Navodi da zna za oštećenje jednog srčanog zaliska i da se nalazi na listi čekanja za kardiohirurški operativni zahvat. Kako navodi, zbog čestih ekstrasistola, redovno koristi tablete bisoprolola 2,5 mg. Strastveni je pušač 14 godina unazad. Socio-epidemiološka anamneza bila je uredna.

Uvidom u medicinsku dokumentaciju, na ultrazvučnom pregledu srca urađenom prethodne godine, uočene su značajno dilatirane leve srčane šupljine uredne kinetike (leva komora 7,9/4,3 cm; leva pretkomora 5,1 × 5,4 × 5,6 cm), uz očuvanu ejekcionu frakciju (EF) leve komore od 75%, masivni prolaps zadnjeg mitralnog kuspisa i degenerativno izmenjenu aortnu valvulu. U dostavljenim laboratorijskim analizama krvi izdvaja se leukocitoza 16,3 hiljada leukocita (granična vrednost 10 hiljada) sa limfocitopenijom od 5,8% (granična vrednost 15%), C - reaktivnim proteinom (CRP) 75,8 mg/l (granična vrednost 0-3 mg/l), laktat-dehidrogenazom (LDH) 420 Ul (granična vrednost 85-227 Ul), d-dimerom 8,5 mg/l FEU (granična vrednost <0,5 mg/l FEU). Na radiografskom snimku pluća zapažaju se masivne mrljaste konfluentne konsolidacije obostrano, skoro zbrisanih ivičnih kontura srca, koje je značajno uvećano.

Kliničkim pregledom uočeno je da je pacijent astenične konstitucije, normalne uhranjenosti, bledo prebojene kože, blago lividnih usana i akralnih završetaka, dispnoičan, tahikardičan (104 otkucaja/min), normotenzivan (krvni pritisak (TA) 110/56 mmHg), subfebrilan (37,4°C), saturacije arterijske krvi (SpO_2 94%) na sobnom vazduhu, određeno pulsnom oksimetrijom. Auskultacijom je utvrđen difuzno oslabljen disajni šum i prisutvo sistolno-dijastolnog šuma nad celim prekordijumom.

Nakon razgovora sa pacijentom i obavljenog kliničkog pregleda, uzet je uzorak venske krvi za određivanje krvne slike, biohemisjkih i hemostatskih parametara, vrednosti CRP, troponina i feritina, prema protokolu. Takođe, uzet je i nazofaringealni bris (NFB) za PCR (engl. *Polomerase Chain Reaction*) dijagnostiku Kovid-19. Predložena je hospitalizacija pacijenta na kliničkom odeljenju uz protokolom propisanu terapiju: ceftriakson 2 g × 2 intravenski (i.v.), hlorokin 500 mg × 2 oralno (p.o.), clexane 0,6 ml × 2 subkutano (s.c.), vitaminsku terapija i.v. (vitamin C 2 g, α-D3 2 mcg), kiseonična potpora 6 l/min.

Na urađenoj kompjuterizovanoj tomografiji (engl. C*omputeed Tomography* - CT) grudnog koša prvog dana hospitalizacije, u parenhimu pluća utvrđene su radiološke karakteristike specifične za uznapredovali stadijum Kovid-19 infekcije, dok se u desnoj pleuri uočava izliv 13 mm i perikardni izliv oko desnog srca od 12 mm. Nakon urađenog CT pregleda, verifikovan je paroksizam supraventrikularne tahikardije (PSVT) sa srčanom frekvencijom 180 otkucaja/min (engl. *Heart Rate* - HR). Srčani ritam konvertovan je intravenskom (i.v) primenom verapamila.

Odlučeno je se da se zbog radiografskog nalaza i ritmološke nestabilnosti pacijenta dalje lečenje nastavi u jedinici intenzivnog lečenja (JIL). Na prijemu u JIL pacijent je budan, komunikativan, dispnoičan i ortopnoičan, preznojen, bledo prebojene kože, cijanotičnih akrosa, HR 110/min, TA 115/60 mmHg, SpO_2 92% uz kiseoničnu potporu preko maske. Bez perifernih edema. Vene vrata nisu nabrekle. U postojeću terapiju uključen je furosemid i.v. 20 mg/12h, nastavljena je kiseonična potpora 5 l/min preko maske uz postignutu SpO_2 92-98%. U arterijskim gasnim analizama (ABG), parcijalni pritisak kiseonika (pO_2) 8,6 kPa, uz fiziološki pH, normokarbiju i zadovoljavajući metabolički status, ali blagu hiperlaktatemiju od 2,4. Rezultat PCR testiranja na Kovid-19 iz prijemne ambulante klinike bio je negativan. Odmah je ponovljen NFB. U laboratorijskim analizama i dalje je prisutna leukocitoza 16,8 hiljada (limfocitopenija 9,3%), LDH 480 U/l, fibrinogen 6,5 g/l (granična vrednost 1,8-3,5 g/l), d-dimer 8,1 mg/l FEU, troponin 18 ng/ml (granična vrednost 14 ng/ml), proBNP 1300 μg (granična vrednost <300 μg/ml), CRP 75,3 mg/l, interleukin 6 (Il-6) 50,0 pg/ml (granična vrednost 0-7 pg/ml). Ponovljena radiografija pluća ukazuje na prisustvo mrljastih konsolidacija obostrano, što klinički u komparaciji sa laboratorijskim nalazima ukazuje na infekciju koronavirusom (Slika 1). Konsultovan je kardiolog koji je predložio dvojnu diuretsku terapiju (furosemid 20 mg/2 h i.v., spironolacton 50 mg/24h p.o.).

four days before with nausea and cough, and then heavy breathing and high temperature. During that time, he used the symptomatic therapy. He negated the existence of allergies to drugs and previous surgical procedures. He stated that he knew about the damage of one heart valve and that he was on the list and waited for the cardio surgical procedure. As he stated, due to frequent extrasystoles, he regularly used bisoprolol tablets 2.5 mg. He has been a passionate smoker for 14 years. Socio-epidemiological anamnesis was neat.

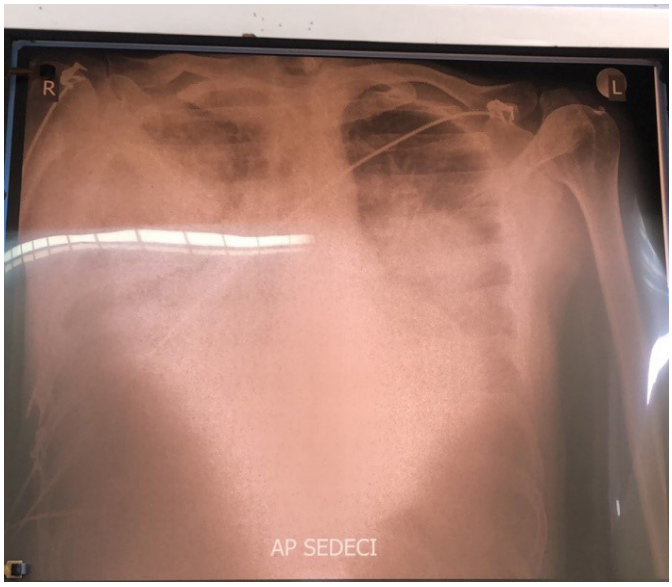
According to the medical documentation, the ultrasound heart examination from the previous year showed the significantly dilated left heart ventricle of neat kinetics (left ventricle 7.9/4.3 cm; left auricle 5.1 × 5.4 × 5.6 cm), with the preserved ejection fraction (EF) of the left ventricle of 75%, massive prolapse of the anterior mitral cusp and degeneratively changed aortic valve. In the laboratory blood findings, leukocytosis of 16.3 thousand leucocytes was found (cut-off 10 thousand) with lymphocytopenia of 5.8% (cut-off 15%), C-reactive protein (CRP) 75.8 mg/l (cut-off 0-3 mg/l), lactate dehydrogenase (LDH) 420 Ul (cut-off 85-227 Ul), d-dimer 8.5 mg/l FEU (cut-off <0.5 mg/l FEU). Massive stained confluent configurations were found on both sides of the lungs on the radiograph image, with almost erased edge contours of the heart, which was significantly enlarged.

During the clinical examination it was noticed that the patient had an asthenic constitution, normal weight, his skin was pale, with mildly livid lips and acral endings, he was dyspnoic, tachycardic 104/min, normotensive 110/56 mmHg, subfebrile 37.4°C, with the saturation of arterial blood (SpO_2) 94% at room temperature, determined by pulse oximetry. Diffusely decreased breath murmur and the presence of systolic-diastolic murmur over the whole precordium were determined by auscultation.

After clinical examination and conversation with the patient, the blood sample was taken from the venous blood in order to determine the biochemical and hemostatic parameters, CRP values, troponin, ferritin, according to the protocol. Also, the nasopharyngeal swab was taken for the PCR diagnostics of Covid-19. The hospitalization of this patient was proposed at the clinic together with the therapy according to the protocol: ceftriaxone 2g × 2 intravenously (i.v., chloroquine 500 mg × 2 orally (p.o.), clexane 0.6 ml x 2 subcutaneously (s.c.), vitamin therapy iv (vitamin C 2 g, α-D3 2 mcg), oxygen support 6 l/min (O_2 support).

The computed tomography (CT) of the chest during the first day of hospitalization, radiological characteristics specific for the late stadium of Covid-19 infection were found in the lung parenchyma, while the right-sided pleural effusion of 13 mm was noticed, as well as the pericardial effusion of 12 mm around the right heart. After CT had been done, paroxysmal supraventricular tachycardia (PSVT) with the heart rate 180/min was verified. Heart rate was treated by the administration of verapamil iv.

The decision was made that the patient should be treated in the intensive care unit due to the radiographic findings and rhythmic instability of the patient. During the admission to the intensive care unit, the patient was awake, communicative, dyspnoic, orthopnoic, sweaty, pale, with acrocyanosis, HR 110/min, TA 115/60 mmHg, SpO_2 92% with oxygen support with the help of a mask. He was without peripheral edema. The neck veins were not swollen. Furosemide i.v. 20 mg/12h was introduced into the existing therapy; he continued to get oxygen support 5 l/min with the help of a mask and SpO_2 was 92-98%. In arterial blood gas analyses (ABG), partial oxygen pressure (pO_2) was 8.6 kPa, with physiological pH, normocarbia, and satisfying metabolic status, but mild hyperlactatemia 2.4. The result of PCR testing for Covid-19 from the infirmary was negative. NFS was immediately repeated. Leukocytosis 16.8 thousand (lymphocytopenia 9.3%), LDH 480 U/l, fibrinogen 6.5 g/l, d-dimer 8.1 mg/l FEU, troponin 18 ng/ml (cut-off 14 ng/ml) proBNP 1300 μg (cut-off < 300 μg/ml), CRP 75.3 mg/l, interleukin 6 (Il-6) 50.0 pg/ml (cut-off 0-7 pg/ml) were still present in the laboratory findings. The repeated lung radiography pointed to the presence of stained bilateral consolidations, which clinically in combination with laboratory findings pointed to the infection of coronavirus (Picture 1). We consulted a cardiologist who proposed a double diuretic therapy (furosemide 20 mg/12h iv, spironolactone 50 mg/24h p.o.).

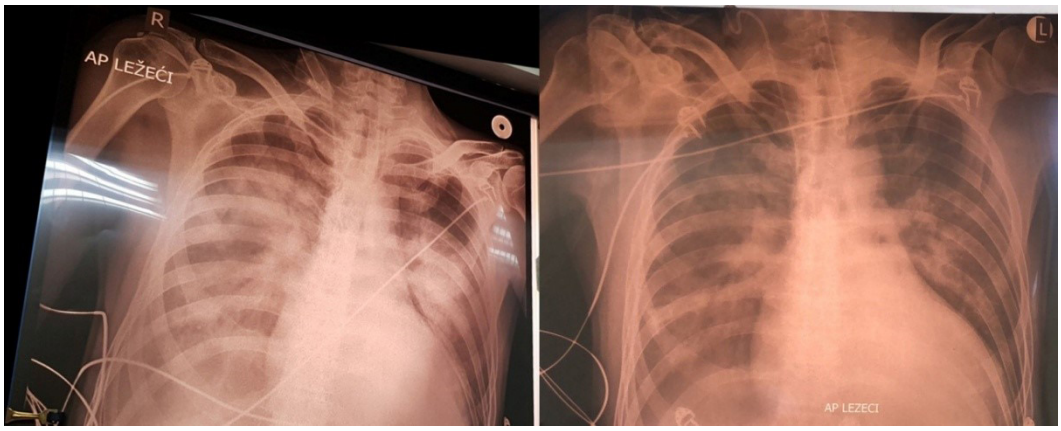


Slika 1. Radiografski snimak pluća na prijemu u jedinicu intenzivnog lečenja

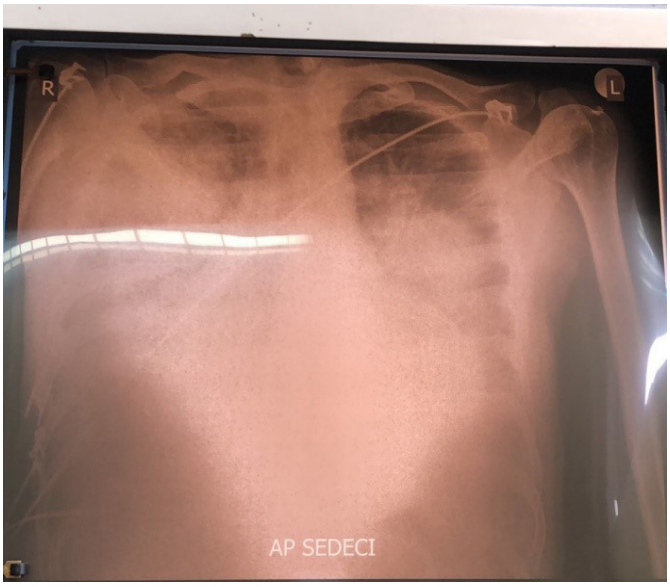
Savetovao je da se dodatna kontrola srčanog ritma sprovodi amiodaronom i presololom uz tablete bisoprolola, uz praćenje dužine QT intervala. U načinjenom EKG zapisu u momentu kardiološkog pregleda, nije bilo poremećaja srčanog ritma, ali su viđeni znaci rane repolarizacije u dijafragmalnim odvodima.

Narednog dana hospitalizacije, pacijent postaje izrazitije dispnoičan, povremeno uznemiren uz osećaj nedostatka vazduha, SpO_2 85% uprkos kiseoničnoj potpori preko maske od 12 l/min, uz očuvanu stabilnost hemodinamike i srčanog ritma. Kiseonična potpora preko maske zamenjena je uređajem sa visokim protokom kiseonika, HFNC (engl. *High Flow Nasal Cannula*). Parametri na HFNC podešeni su na inspiratornu frakciju kiseonika (FiO_2 60%), protok 40 l/min, čime se SpO_2 koriguje na 90-95%. Pristigao je rezultat ponovljenog PCR testiranja koji je opet negativan. U toku večernjih sati, ponovo PSVT 180/min, kupirana primenom verapamila i.v. Iz terapije je isključen hlorokin. Tokom noći, pacijent je bio respiratorno i kardiovaskularno stabilan.

Narednog dana, u jutranjim satima, pacijent je psihomotorno uznemiren, dezorijentisan, otežano kooperativan, hiposaturisan (SpO_2 90%) uprkos intenzivnoj kiseoničnoj potpori, očuvanih hemodinamskih parametara. Odlučuje se da se kod pacijenta primeni neinvazivna ventilatorna potpora (NIMV): CPAP-PS mod (FiO_2 60%, PEEP 5 cmH_2O, PS 12 cmH_2O), sa *full face* maskom. Promenjeni režim potpore je poboljšao oksigenaciju, ali je pacijent kratkotrajno toleriše,



Slika 2. Radiografski snimak pluća tokom perioda primene invazivne ventilatorne potpore (levo - 4h nakon intubacije, desno - neposredno pre ekstubacije)

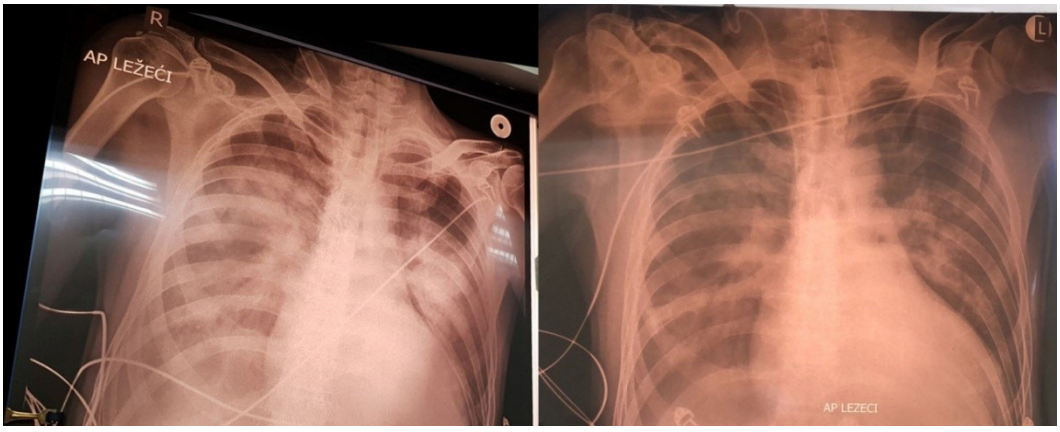


Picture 1. Radiographic image of lungs during admission at the intensive care unit

He advised controlling the cardiac rhythm additionally with amiodarone and presolol together with tablets of bisoprolol, and observation of length of QT intervals. The ECG recording during the cardiological examination did not show disorders of heart rhythm, but signs of early repolarization in diaphragm drains were noticed.

During the next day of hospitalization the patient became more dyspnoic, and at times anxious and lacking air, SpO_2 85%, in spite of oxygen support using a mask of 12 l/min, with preserved stability of hemodynamics and heart rate. Oxygen support with the help of a mask was replaced by the high flow nasal cannula (HFNC). Parameters on the HFNC were adjusted for the fraction of inspired oxygen (FiO_2 60%), the flow 40 l/min, by which SpO_2 was 90-95%. The result of the repeated PCR testing was negative again. During evening hours, PSVT was 180/min and decreased by verapamil iv. Chloroquine was eliminated from the therapy. During the night, the patient's respiratory and cardiovascular state was stable.

The next day, in the morning hours, psychomotor agitation was present, he was disoriented, cooperated with difficulty, hyposaturated (SpO_2 90%) in spite of intensive oxygen support, with preserved hemodynamic parameters. The decision was made to administer the non-invasive ventilatory support: CPAP-PS mod (FiO_2 60%, PEEP 5 cmH_2O, PS 12 cmH_2O) with the full face mask. The changed regime of support improved oxygenation, but the patient tolerated



Picture 2. Radiographic image of lungs during the period of application of invasive ventilatory support (left – 4h after intubation, right – immediately before extubation)

zbog čega je odlučeno da se pacijent intubira. U nastavku, za potrebe intubacije, pacijent je sediran midazolamom 10 mg i.v., propofolom 50 mg i.v., esmeronom 80 mg (1 mg/kg) i.v., intubiran, nakon čega je obezbeđena mehanička ventilatorna potpora - MVP (*BiLevel mod*: FiO2 50%, *Plow* 5 cm H2O, P*high* 15 cmH2O, RR 12/min, I:E- 1:2). Plasiran je centralni venski kateter preko desne jugularne vene, arterijska kanila preko *a. radialis dex*. i obezbeđeno invazivno merenje arterijskog krvnog pritiska. Naknadno je obezbeđena kontinuirana sedacija midazolamom (0,1 mg/kg/h i.v.) i vazopresorna potpora cirkulacije noradrenalinom (0,03 mcg/kg/min i.v.) uz postojeću dvojnu diuretsku terapiju. Plasirana je nazogastrična sonda zbog ishrane i primene oralne terapije. Urađene ABG ukazuju na dobru gasnu razmenu i uredan metabolički status (pH 7,42/ pO_2 10,2 kPa/ pCO_2 5,3kPa/ sO_2 95%/ HCO_3 22,3/ BEecf-1,2/ Lac 1,4). Urađena je radiografija pluća četiri sata nakon intubacije (Slika 2, levo) koja ukazuje na značajno manju konsolidaciju sa desne strane uz održavanje konsolidacije sa leve strane u srednjem plućnom polju, u poređenju sa grafijom od prethodnog dana. Pacijent je i dalje subfebrilan (37,3°C), te zamenjena antibiotska terapija: isključen ceftriakson, uključen cefepim 1 g/12h i.v., levofloxacin 500 mg/24h i.v., fluconazol 400 mg/24h. Kako je NFB dvostruko negativan, odlučuje se da se NFB ponovi za Kovid-19 i uzorkuje bronho-alveolarni lavat (BAL) i uradi PCR testiranje na proširenu paletu respiratornih virusa i Kovid-19, koji bi mogli dati sliku intersticijalne pneumonije i akutnog respiratornog distres sindroma (engl. *Acute Respiratory Distress Syndrome* - ARDS). Uzeti su uzorci krvi za hemokulturu (iz plasiranog centralnog venskog katetera i iz periferne vene) i uzorak urina za urinokulturu.

Narednog dana, pacijent je i dalje kontinuirano sediran midazolamom, na poziv otvara oči i izvršava jednostavne naloge. Intubiran, na MVP (CPAP-PS: FiO2 50%, PEEP

Tabela 1. Paleta mogućih prouzrokovača respiratornog distresa (rezultat)

Vrsta uzorka: **Nazofaringealni bris**	**Lab. broj protokola:** **XXXX**	**Uzorkovan: 20.04.2020.** **Primljen: 20.04.2020. 01:08**
Napomena	PCR respiratorni panel:	
Adenovirus	Nije detektovana DNK	
Coronavirus 229E	Nije detektovana RNK	
Coronavirus HKU1	Nije detektovana RNK	
Coronavirus NL63	Nije detektovana RNK	
Coronavirus OC43	Nije detektovana RNK	
Coronavirus (MERS-CoV)	Nije detektovana RNK	
Metapneumovirus-humani	Nije detektovana RNK	
Rhinovirus-humani/Enterovirus	Nije detektovana RNK	
Influenza A	Nije detektovana RNK	
Influenza B	Nije detektovana RNK	
Parainfluenza virus 1	Nije detektovana RNK	
Parainfluenza virus 2	Nije detektovana RNK	
Parainfluenza virus 3	Nije detektovana RNK	
Parainfluenza virus 4	Nije detektovana RNK	
Parainfluenza virus 1	Nije detektovana RNK	
Resiratorni sincicijalni virus	Nije detektovana RNK	
Bordatella pertussis	Nije detektovana DNK	
Bordatella parapertussis	Nije detektovana DNK	
Chlamydophila pneumoniae	Nije detektovana DNK	
Mycoplasma pneumoniae	Nije detektovana DNK	

it for a short time, and therefore, the decision was made to intubate him. For the needs of intubation, the patient was sedated with midazolam 10 mg i.v., propofol 50 mg i.v., Esmeron 80 mg (1 mg/kg), intubated, and then mechanical ventilatory support was provided – MVS (BiLevel mod: FiO_2 50%, Plow 5 cmH_2O, RR 12/min, I:E-1:2). The central venous catheter was placed via the right jugular vein, arterial cannula via *a.radialis.dex.* and invasive measuring of arterial blood pressure was provided. Continuous sedation with midazolam was provided (0.1 mg/kg/h i.v.) as well as vasopressor support of circulation with noradrenaline (0.03 mcg/kg/min i.v.) with the existing double diuretic therapy. A nasogastric tube was placed for oral therapy and food. ABG pointed to the good gas exchange and neat metabolic status (pH 7.42/pO_2 10.2kPa/pCO_2 5.3kPa/ sO_2 95%/HCO_3 22.3/BEecf-1.2/Lac 1.4). The lung radiography was done 4 hours after the intubation (Picture 2, on the left), which pointed to the significantly decreased consolidation on the right side with the consolidation on the left side in the mid-lung, in comparison to radiography from the previous day. The patient was still subfebrile (37.3°C), and therefore antibiotic therapy was changed: ceftriaxone was not administered, and cefepime was introduced 1 g/12h i.v., levofloxacin 500 mg/24h i.v., fluconazole 400 mg/24h. As NFS was negative two times, the decision was made to take the NFS for Covid-19 again and to take a sample for broncho-alveolar lavage (BAL) and do the PCR test for the expanded range of respiratory viruses and Covid-19, as well, which would give a picture of interstitial pneumonia and acute respiratory distress syndrome. Blood samples were taken for hemoculture (from the placed central venous catheter and from the peripheral vein) and the sample of urine for urine culture.

The next day, the patient was continuously sedated by midazolam, he opened eyes when he was asked to and reacted to simple orders.

Table 1. A list of possible causes of respiratory distress (result)

Type of sample: Nasopharyngeal swab	**Laboratory protocol number: XXXX**	**Sampled: 20.04.2020. Received: 20.04.2020. 01:08**
Note	PCR respiratory panel:	
Adenovirus	No DNA was detected	
Coronavirus 229E	No RNA was detected	
Coronavirus HKU1	No RNA was detected	
Coronavirus NL63	No RNA was detected	
Coronavirus OC43	No RNA was detected	
Coronavirus (MERS-CoV)	No RNA was detected	
Human metapneumovirus	No RNA was detected	
Rhinovirus-human/Enterovirus	No RNA was detected	
Influenza A	No RNA was detected	
Influenza B	No RNA was detected	
Parainfluenza virus 1	No RNA was detected	
Parainfluenza virus 2	No RNA was detected	
Parainfluenza virus 3	No RNA was detected	
Parainfluenza virus 4	No RNA was detected	
Parainfluenza virus 1	No RNA was detected	
Respiratory syncytial virus	No RNA was detected	
Bordatella pertussis	No DNA was detected	
Bordatella parapertussis	No DNA was detected	
Chlamydophila pneumoniae	No DNA was detected	
Mycoplasma pneumoniae	No DNA was detected	

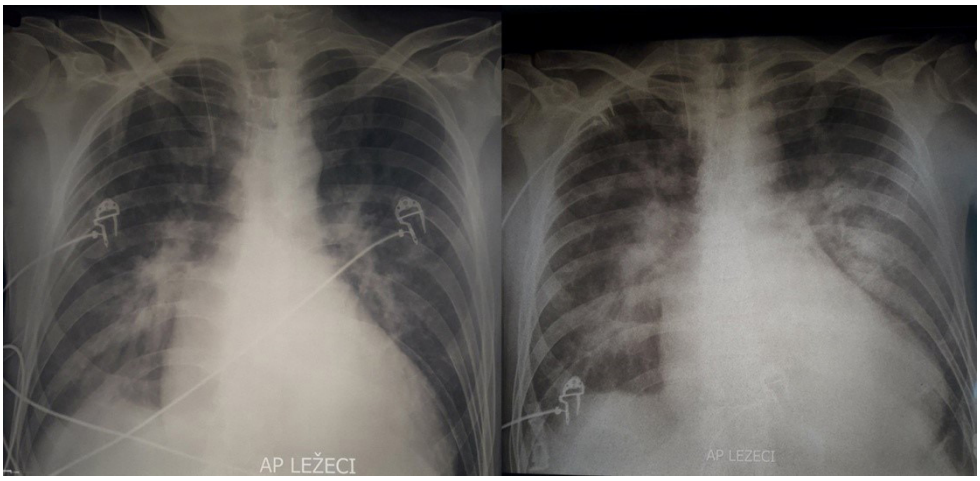
5, PS 10), uredne gasne razmene i metaboličkog statusa, afebrilan. Hemodinamika je održavana minimalnom dozom noradrenalina (0,01 mcg/kg/min), a diureza je stimulisana dvojnom diuretskom terapijom u istoj dozi. Srčana frekvencija održavana je tabletama bisoprolola. Doza cleksana kontrolisana je merenjem vrednosti anti Xa (0,45-0,62). Laboratorijski, blaga leukocitoza bez odstupanja u leukocitarnoj formuli (leukociti 10,2 hiljade), CRP 5,6 mg/l, Il-6 15,8 pg/ml, LDH 260 U/l, troponin 11 ng/ml, proBNP 980 μg. Tokom večeri, isključena je sedacija midazolamom.

Narednog jutra, ponovljena je radiografija pluća (Slika 2, desno) i kako je nalaz u značajnoj regresiji, a pacijent u potpunosti kooperativan, laboratorijski parametri korektni, uz minimalnu vazopresornu potporu dobro kontrolisana hemodinamika, odlučuje se da se pacijent ekstubira. Nakon ekstubacije, uklonjena je nazogastrična sonda, obezbeđena je kiseonična potpora kiseoničnom maskom (protok 12 l/min), zadržana je vazopresorna potpora cirkulacije i sva prethodna terapija u već postojećim dozama.

Narednog dana, pristigao je rezulatat PCR testiranja koji je bio negativan na sve ispitivane viruse (Slika 3). Takođe, sterilni su bili i uzorci za hemokulturu i urinokulturu. Ponovljena grafija pluća koja je opisana da je bez bitnijih promena u odnosu na prethodnu (Slika 4, levo). Urađen je ultrazvučni pregled srca koji je u odnosu na prethodni ukazao na značajno redukovanu funkciju leve komore, sa EF 35% uz hipokontraktilnost svih zidova leve komore. Konsultovan je kardiohirurg koji je potvrdio neophodnost operativnog lečenja valvularne mane na aortnoj i mitralnoj poziciji, uz prethodnu sanaciju infekcije i kardiološku pripremu. U trenutku pregleda, pacijent je bez indikacija za hitnim operativnim kardiohirurškim lečenjem. Isključena je vazopresorna potpora noradrenalinom, ostala terapija sprovedena je kao prethodnog dana. Kako je pacijent eupnoičan, aktivan u postelji, urednih parametra iz ABG, postepeno je redukovana kiseonična potpora (maska, 6 l/min). Svi parametri zapaljenskog sindroma bili su u referentnom opsegu.

Narednog dana (9. dana lečenja u JIL), odlučeno je da pacijent nastavi lečenje na Odeljenju kardiologije za lečenje srčane insuficijencije KCS. Neposredno pre otpusta iz JIL, urađen je kontrolni RTG snimak pluća koji je opisan kao stacionaran (Slika 4, desno). Na otpustu, pacijent je eupnoičan, na kiseoničnoj potpori preko maske, SpO_2 98%, hemodinamksi i ritmološki stabilan, afebrilan i bez perifernih otoka.

Nakon jednomesečne kardiološke pripreme i optimizacije kadiovaskularnog statusa, započete su pripreme za kardiohirurišku proceduru. Serološki test na SARS-CoV-2 bio je negativan, kao i ostale mikrobiološke pretrage. Koronarografija nije ukazala na postojanje koronarne bolesti. Dva meseca nakon hospitalizacije na Infektivnoj klinici KCS pacijent je preveden na kardiohirurgiju gde je urađen rekonstruktivni kardiohirurški zahvat (zamena ošetećenog zaliska veštačkim na mitralnoj i



Slika 3. Radiografski snimak pluća posle ekstubacije (levo) i na otpustu sa lečenja (desno)

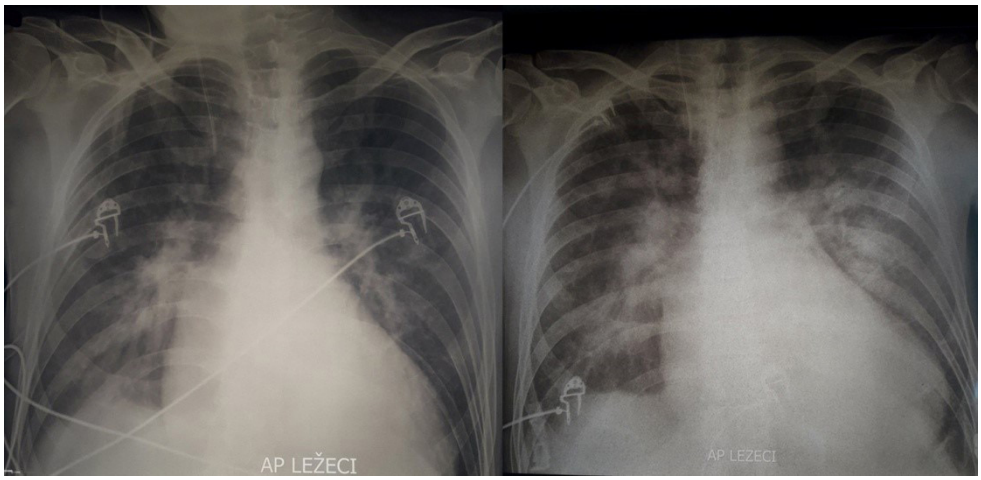
He was intubated, on MVS (CPAP-PS: FiO_2 50%, PEEP 5, PS 10), with regular gas exchange and metabolic status, afebrile. Hemodynamics was regulated by the minimal dose of noradrenaline (0.01 mcg/kg/min), while diuresis was stimulated by double diuretic therapy in the same dose. Heart rate was maintained by tablets of bisoprolol. The dose of clexane was controlled by the measurement of anti-Xa values (0.45-0.62). Laboratory findings showed mild leukocytosis without deviations in leukocyte formula (leukocyte 10.2 thousand), CRP 5.6 mg/l, Il-6 15.8 pg/ml, LDH 260 U/l, troponin 11 ng/ml, proBNP 980 μg. During the evening, sedation with midazolam was stopped.

The next morning, chest radiography was done (Picture 2, on the right) and since the findings showed significant regression and the patient was completely cooperative, laboratory findings were correct, hemodynamics controlled with minimal vasopressor support, the decision was made to extubate the patient. After the extubation, the nasogastric tube was removed and oxygen support was provided via the oxygen mask (flow 12 l/min), vasopressor support of circulation was kept and all the previous therapy in the current doses. The next day, the PCR testing result was negative for all the examined viruses (Picture 3). Also, samples for hemoculture and urine culture were sterile.

The repeated lung radiography was without significant changes in comparison to the previous one (Picture 4, on the left). The ultrasound examination of the heart pointed to the significantly reduced function of the left ventricle, with EF 35% with hypercontractility of all walls of the left ventricle. The cardiac surgeon confirmed that it was necessary to treat surgically the valvular defect on the aortic and mitral position with the previous curing of infection and cardiac surgery preparation. During the examination, there were no indications for urgent cardiac surgery. The vasopressor support with noradrenaline was canceled, while the therapy was the same as the previous day. As the patient was epnoic, active in bed, with regular parameters from ABG, the oxygen support was gradually reduced (mask 6 l/min). All parameters of the inflammatory syndrome were within the reference range.

The next day (9th day at the intensive care unit), the decision was made that the patient should be treated at the Department for Cardiology for the treatment of cardiac insufficiency at the Clinical Centre of Serbia. Before he was discharged from the intensive care unit, the control lung radiography was described as stationary (Picture 4, on the right). At the moment of discharge from the hospital, the patient was eupneic, on the oxygen support via a mask, SpO2 98%, with stable hemodynamics and rhythm, afebrile, without peripheral edema.

After the cardiac surgery preparation that lasted one month and the organization of cardiovascular status, the preparations for the cardiac surgical procedure were made. The serology testing for SARS-CoV-2 was negative, as well as other microbiological findings.



Picture 3. Radiographic image of lungs after extubation (left) and during discharge from the hospital (right)

aortnoj poziciji). Celokupan perioperativni period je protekao bez komplikacija. Mesec dana nakon izvršenog operativnog zahvata, redovna ultrazvučna kontrola ukazuje na EF 35% i dobro funkcionalne veštačke zaliske na mitralnoj i aortnoj poziciji.

Diskusija

Komorbiditet predstavlja nezavistan faktor rizika za razvoj teške kliničke slike Kovida-19 (4). Kako navode Jordan i saradnici (5), najčešći i najzastupljeniji zajednički faktori rizika su starost, pol, gojaznost, dijabetes melitus, hipertenzija, bolest kardiovaskularnog sistema i hronična bolest pluća (astma i hronični opstruktivni bronhitis). Liang navodi da nije dokazana jasna korelacija između postojanja maligniteta i težine kliničke slike Kovid-19 oboljenja (6). Međutim, Livingston i saradnici ukazuju da značajno mesto imaju faktori rizika kao što su muški pol i pušenje cigareta (7). U studiji koju su ovi autori sproveli među italijanskom populacijom, navodi se da pušenje cigareta posledično utiče na obolevanje kardiovaskularnog i respiratornog sistema, potom i na značajno veću mogućnost formiranja teške kliničke slike bolesti. Isti autori navode da je korelacija komorbiditeta i težine kliničke slike Kovid-19 uslovljena i ažurnošću zdravstvenog sistema jedne države i njenih građana da blagovremeno registruju komorbiditete, kako bi se preciznije moglo zaključivati o komorbiditet - klinička slika intereakciji u momentu transmisije virusa.

U opisanom prikazu slučaja, pacijent je imao vrlo ozbiljne faktore rizika za razvoj teškog oblika Kovid-19: muški pol, pušenje cigareta i kardiovaskularni komorbiditet u fazi akutizacije. Međutim, srčana insuficijencija koja je nastupila u fazi epidemije novog korona virusa je najpre posledica prisustva dugogodišnjih valvularnih mana, strukturnih i funkcionalnih promena na miokardu. Intereakcija između Kovida-19 i komorbiditeta je dvosmerna. Kako navode Clerkin i saradnici (8), princip ulaska SARS-CoV- 2 u organizam je vezivanje serin-proteaze 2 virusa za receptor angiotenzin-konvertujućeg enzima 2 (ACE2) čoveka. Ekspresija ACE2 je najveća u plućima (tip 2 alveolarnih ćelija), u vaskularnom endotelu, bubrezima, gastrointestinalnom sistemu i u srcu. Što je veća aktivnost ACE2, to je veća transmisija virusa u organizmu i teža klinička slika. Opisani mehanizam za nastanak bolesti se dešava u stanjima kod kojih postoji pojačana aktivnost ACE2, odnosno povećana aktivnost renin-angiotenzin sistema: ateroskleroza, hipertenzija (naročito kod upotrebe ACE inhibitora zbog ushodne regulacije broja receptora za ACE2), srčana insuficijencija.

Kako navode Shi i saradnici (9,10), u nekoliko studija sprovedenih na velikom uzorku pacijenata, dokazan je uticaj infekcije koronavirusom na kardiovaskularni sistem: direktno oštećenje miokarda, akutni koronarni sindrom, aritmije, arterijski i venski tromboembolizam. Prikazani pacijent je bolovao od srčane insuficijencije sa očuvanom EF, na šta ukazuje ultrazvučni pregled urađen 2019. godine kada je zabeležena EF leve komore 75% uz značajnu dilataciju levih srčanih šupljina. Pacijent nije koristio lekove iz grupe ACE inhibitora u hroničnoj terapiji srčane insuficijencije. Međutim, značajno povišena vrednost proBNP uz vrednosti troponina u referentnom opsegu ukazuje na izostanak akutnog koronarnog sindroma, ali na prisutnu slabost leve komore.

Od samog početka hospitalizacije pacijenta razmatrana je mogućnost pogoršanja srčane funkcije sa ili bez prisustva virusa, zbog čega je urađen CT pregled grudnog koša koji je jasno ukazao na razvijeni oblik Kovid-19 pneumonije. Pravilnim i blagovremenim uzorkovanjem nismo uspeli da dokažemo prisustvo korona virusa (ponavljano PCR testiranje, BAL uzorkovanje) niti prisustvo nekog drugog infektivnog agensa u uzorku urina i krvi, iako laboratorijski parametri ukazuju na postojanje infektivnog procesa (leukocitoza uz tipičnu leukocitarnu formulu, povišen CRP i Il-6, subfebrilnost). Tan i saradnici navode jasnu korelaciju između CT nalaza i vrednosti CRP u stratifikovanju kliničke slike Kovid-19 (11). Isti autori navode nedovoljno dobru korelaciju između CT nalaza i vrednosti limfocita, ističući veliku dijagnostičku senzitivnost CT pregleda. Kako navode Stojadinović i saradnici (12), prema preporukama ekspertskog panela Naučnog odbora časopisa *Radiology* (13), smatra se da je senzitivnost CT pregleda grudnog koša za dijagnozu Kovid-19 pneumonije 80-90%,

Coronarography did not point to the existence of a coronary disease. Two months after the hospitalization at the Clinic for Infectious Diseases of the Clinical Centre of Serbia, the patient was moved to cardiac surgery, where the reconstructive cardiac surgical procedure was done (replacement of the damaged heart valve with the artificial one on the mitral and aortic position). The whole perioperative period passed without complications. One month after the surgical procedure, regular ultrasound check-up showed that EF was 35% and that artificial valves on mitral and aortic positions functioned well.

Discussion

Comorbidity presents an independent risk factor for the development of a severe clinical picture of Covid-19 (4). According to Jordan and associates (5), the most frequent and most common risk factors are age, gender, obesity, diabetes mellitus, hypertension, diseases of the cardiovascular system, and chronic lung diseases (asthma and chronic obstructive bronchitis). Liang states that the clear correlation between the existence of malignancy and the severity of the clinical picture of Covid-19 has not been proved (6). However, Livingston and associates state that significant risk factors are male gender and smoking cigarettes (7). In the study conducted by these authors in the Italian population, they state how smoking consequently induces cardiovascular and respiratory diseases, and then the possibility of severe forms of the disease. The same authors state that the correlation of comorbidities and the severity of clinical picture of Covid-19 is conditioned by the agility of the health care system of one state and its citizens to register the comorbidities on time so that conclusions could be made on the comorbidities, that is, the interaction between the comorbidities and clinical picture at the moment of virus transmission.

In the described case report, the patient had very pronounced risk factors for the development of severe forms of Covid-19: male gender, smoking and cardiovascular comorbidity in the acute phase. However, cardiac insufficiency, which appeared during the epidemic of the novel coronavirus, is the consequence of valve deficiencies of many years, structural and functional changes of the myocardium. The interaction between Covid-19 and comorbidities is a two-way relationship. As Clerkin and associates (8) state, the principle of entry of SARS-CoV-2 into the organism is the binding of serin-protease 2 virus to the receptor of the angiotensin-converting enzyme 2 (ACE2). The expression of ACE2 is greatest in the lungs (type 2 alveolar cells), in the vascular endothelium, kidneys, gastrointestinal system, and heart. When the activity of ACE2 is greater, the virus transmission in the organism is bigger and the clinical picture is more severe. The described mechanism for the appearance of this disease happens when the activity of ACE2 increases, that is, the activity of the rennin-angiotensin system: atherosclerosis, hypertension (especially when ACE inhibitors are used due to the rising regulation of the number of receptors for ACE2), cardiac insufficiency.

According to Shi (9,10), in a few studies conducted on a large sample of patients, the influence of novel coronavirus on the cardiovascular system has been proven: direct damage of myocardium, acute coronary syndrome, arrhythmias, arterial and venous thromboembolism. The patient, who we presented, suffered from cardiac insufficiency with the preserved EF, to which the ultrasound examination from December 2019 pointed, when EF of the left ventricle was 75% with significant dilatation of left heart cavities. The patient did not use drugs from the group of ACE inhibitors in the chronic therapy of cardiac insufficiency. However, a significantly increased level of proBNP in addition to troponin values in the reference range pointed to the absence of acute coronary syndrome, but to the present weakness of the left ventricle.

From the very beginning of the patient's hospitalization, the possibility of worsening of the cardiac function was considered with or without the virus, and therefore CT of the chest was done and it pointed clearly to the developed form of Covid-19 pneumonia. Timely and correct sampling did not prove the presence of coronavirus (repeated PCR testing, BAL sampling) or some other infectious agents in the blood and urine samples, although laboratory parameters pointed to the presence

a specifičnost 60-70%. Oni preporučuju CT pregled u situacijama ukada treba doneti odluku o daljem lečenju ili kada je neophodno razlikovanje Kovid-19 pneumonije od pneumonije druge etiologije.

I dalje ostaje nejasno zašto nismo uspeli da dobijemo pozitivan PCR test za ovog bolesnika iako smo uzorak uzeli i iz BAL-a, a celokupna klinička slika i laboratorijski parametri su govorili u prilog postojanja korona virus infekcije i posledičnog srčanog popuštanja. Ovaj prikaz pokazuje svu komplikovanost u lečenju i dijagnostici pacijenata sa potencijalnom Kovid-19 infekcijom, a posebno kod pacijenata sa pratećim oboljenjima.

Zaključak

Prikazom kliničkog toka bolesti, dijagnostičkog i terapijskog pristupa kod pacijenta sa kardiovaskularnim komorbiditetom hteli smo da ukažemo i na poteškoće u prepoznavanju kliničke slike i dijagnostikovanju Kovid-19. Ovaj prikaz slučaja sugeriše na neophodnu organizacionu i dijagnostičku stratifikaciju pacijenata sa komorbiditetima koji se manifestuju kliničkom i laboratorijskom slikom koja je slična Kovidu-19. Kako bismo smanjili rizik da pacijent sa pogoršanjem hronične bolesti bude dodatno životno ugrožen, neophodno je pacijente sa komorbiditetima primarno hospitalno lečiti na formiranim odeljenjima za izolaciju u okviru nekovid bolnica.

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of infectious process (leukocytosis for the typical leukocyte formula, increased CRP and Il-6, subfebrile state). Tan and associates state the clear correlation between CT findings and CRP values in the stratification of the clinical picture of Covid-19 infection (11). The same authors state that the correlation between CT findings and lymphocyte values is not sufficient, emphasizing the great diagnostic sensitivity of CT findings. As Stojadinovic and associates state (12), according to the recommendations of the panel of experts from the scientific board of Radiology journal (13), the sensitivity of CT chest examination for the diagnosis of Covid-19 pneumonia is considered to be 80-90%, while specificity is 60-70%. They recommend the CT examination in situations, in which it will influence the decision on further treatment, as well as when it is necessary to differentiate Covid-19 pneumonia from pneumonia of different etiology.

It is still not clear why we did not get the positive PCR test for this patient although we took the sample from the BAL as well, and the whole clinical picture and laboratory findings spoke in favor of coronavirus infection and resulting cardiac insufficiency. This report presents all the complexities in relation to the treatment and diagnostics of patients with potential Covid-19, and especially in patients with comorbidities.

Conclusion

The aim of this presentation of the clinical course of this disease, diagnostic and therapeutic approach in the patient with cardiovascular comorbidities was to point to the difficulties in recognizing the clinical picture in Covid-19 diagnostics. This case report suggests that the organizational and diagnostic stratification of patients with comorbidities, which are manifested as clinical and laboratory picture similar to Covid-19, is necessary. In order to diminish the risk that is additionally life-threatening to patients with the worsening of chronic diseases, it is necessary to treat patients with comorbidities primarily at hospitals within specially created departments for isolation in non-Covid hospitals.

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SPECIFIČNOSTI ADOLESCENTNE KRIZE U VREME PADEMIJE KOVID-19

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SAŽETAK

Uvod/Cilj: Bez obzira u kojoj fazi adolescencije se nalaze, adolescenti kroz akcidentalnu krizu prolaze kroz tri faze: negaciju realne opasnosti; osećaj zbunjenosti i haosa sa početkom ispoljavanja prvih simptoma i potpuni depresivni slom sa različitim spektrom manifestovanja. Kovid-19 pandemija je postala realan izazov za ove pacijente. Cilj istraživanja je prikaz i analiza kliničkog psihoterapijskog materijala opisanih faza prolaska kroz akcidentalnu krizu, izazvanu pandemijom Kovid-19, kod adolescenata različitog uzrasta.
Metode: U radu su korišćeni materijali psihoterapijskih seansi adolescenata različith faza adolescentne krize. Za objašnjenje negacije realne opsnosti prikazani su adolescenti rane i središne faze adolescencije, a za osećaj zbunjenosti i haotičnosti, do potpunog depresivnog sloma, adolescenti kasne i postadolescentne faze.
Prikaz pacijenata: Kod adolescenata, koji nisu ispoljavali problematičan prolazak kroz razvojnu krizu, akcidentalna kriza pandemije dovodi do negiranja realne opasnosti. Kod mlađe, dvanaestogodišnje adolescentkinje, na konkretnom nivou funkcionisanja, dolazi do oslabljene koncentracije za učenje. Kod nešto starijeg, petnaestogodišnjeg adolescenta, negiranje realne opasnosti, kroz sublimaciju, prelazi u alturizam. Kod adolescenata sa već ispoljenim problematičnim prolaskom kroz razvojnu krizu, dolazi do pogoršanja simptoma usled dejstva superponirane akcidentalne krize izazvane pandemijom. Kod adolescentkinje središne faze adolescencije, dolazi do mešanja realnih somatskih problema sa somatizacijom. Kod starijeg adolescenta, postadolescentne faze, sa problemima bolesti zavisnosti, pandemija provocira iritabilnost i nisku frustrativnu toleranciju, dovodeći do potpunog depresivnog sloma.
Zaključak: Superponiranje akcidentnalnih kriza uvek komplikuje normativnu adolescentnu krizu, ali naglašavamo da ispoljena psihopatologija može biti prolaznog karaktera. Psihijatrijsko psihoterapijska intervencija određuje se u odnosu na stepen preteće ili kompletno ispoljene dekompenzacije i može se kretati od kraćih savetodavnih intervencija, do intenzivnih ozbiljnih psihoterapija, uz pomoć ili bez adekvatne medikamentozne terapije.

Ključne reči: adolescentna kriza, pandemija, Kovid-19

Uvod

Iako se adolescencija zapaža još u doba industrijske revolucije, u XIX veku, do njenog potpunog pravilnog pozicioniranja u psihijatrijsko psihološkom razumevanju dolazi tek pedesetih godina prošlog veka, kada dobija status razvojno normativne krize (1). To je najosetljiviji, ali i najvažniji period u životu kada se svi konflikti i sve nedorečenosti ponovo proživljavaju. Pojavom socijalne psihijatrije, definišu se pojmovi razvojne i akcidentalne krize (2). Kroz razvojne krize prolazimo svi, a kroz akcidentalne samo oni koje akcidenti u životu zadese. Mogućnost istovremnog proživljavanja akcidentalne krize i razvojno normativne krize, uvek je potencijalna opasnost (3). Zvanična psihijatrija dugo je ostala zatvorena za razumevanje adolescencije, poznavajući prvo samo odrasle, zatim odrasle i decu (4). Celovito sagledavanje adolescencije traje i danas, sa perzistiranjem terminološke konfuzije pojmova omladina, pubertet i adolescencija. Omladinu proučavaju istoričari i sociolozi. Pubertetske promene se izučavaju od najstarijeg doba, ali u smislu biološko-fizioloških promena, najčešće od strane pedijatra. Adolescencija je period o kome govore kliničari psihoterapeuti: psihijatri i psiholozi (4,5). Frojdova psihoanalitička misao

CASE REPORT

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THE SPECIFICS OF ADOLESCENT CRISIS DURING THE COVID-19 PANDEMIC

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SUMMARY

Introduction/Aim: Adolescents pass through three phases of an accidental crisis: the negation of real danger, feeling of confusion and chaos with the onset of the first symptoms, a complete depressive breakdown with a different range of manifestations. The Covid-19 pandemic has become a real challenge for these patients. The aim of this study is to review and analyze the clinical psychotherapeutic material of the described phases of the passage through the accidental crisis, caused by the Coronavirus pandemic.
Methods: Adolescents of different ages are involved. The materials of psychotherapeutic sessions of adolescents were used in the paper, from different phases of the adolescent crisis. To explain the phase of negation of real danger, adolescents of the early and central stages of a period of adolescence are presented. To explain the phase of feelings of confusion and chaos to complete depressive breakdown, adolescents of the late and post adolescent stages of a period of adolescence are presented.
Patient presentation: In adolescents, who did not show a problematic passage through the developmental crisis, the accidental crisis of the pandemic leads to the denial of the real danger. In a younger, twelve-year-old adolescent, at a specific level of functioning, there is a weakened concentration for learning. In a slightly older, fifteen-year-old adolescent, the denial of real danger, through sublimation, goes into altruism. In adolescents with already manifested problematic passage through the developmental crisis, the symptoms worsen due to the effect of the superimposed accidental crisis caused by the pandemic. In adolescents, the middle phase of adolescence, there is a mixture of real somatic problems with somatization. In the older adolescent, post adolescent phase, with addiction disease problems, the pandemic provokes irritability and low frustrating tolerance leading to complete depressive breakdown.
Conclusion: We can conclude that the superposition of accidental crises always complicates the normative adolescent crisis, but we emphasize that the manifested psychopathology can be of a transient character. Psychiatric psychotherapeutic interventions are determined in relation to the degree of threatening or completely manifested decompensation from shorter counseling interventions to the intensification of deep psychotherapy, with or without adequate drug therapy.

Key words: adolescent's crisis, pandemic, Covid-19

Introduction

Although the notion of adolescence was noted in the times of industrial revolution in the 19th century, it was completely and correctly positioned in the psychiatric-psychological field in the 1950s, when it was given the status of developmental normative crisis (1). It is the most vulnerable, and also the most important period in life, when all conflicts and unsaid words are experienced once again. With the appearance of social psychiatry, the notions of the developmental and accidental crises were defined (2). All people pass through developmental crises, while accidental crises are experienced only by those people who are involved in accidents. The possibility of undergoing the accidental crisis and developmental normative crisis at the same time is always a potential danger (3). Official psychiatry has been closed for understanding adolescence for a long time, knowing only about adults at first, and then adults and children (4). A comprehensive understanding of adolescence has persisted to these days, with the terminological confusion of terms such as youth, puberty, and adolescence. The notion

daje prva razumevanja adolescencije definišući je kao prelazni period između detinjstva i odraslog doba. Karakterišu je intezivne emotivne bure, učestala i brza promena raspoloženja i suočavanje sa probuđenim edipalnim sukobima (5).

Adolescencija dobija veću pažnju u teoriji ličnosti psihijatra-psihoanalitičara E. Eriksona, po kojoj je ceo život podeljen na osam očekivanih razvojno normativnih kriza, gde adolescenciji pripada centralno mesto pete normativne krize (1). Ponovo je naglašeno da je adolescencija najburnija kriza u životu u kojoj se prelamaju svi problemi i sva, i dobra i loša, rešenja iz ranijih životnih faza, sa ciljem sintetizovanja dotadašnjih životnih iskustava i izgradnje pouzdanog i stabilnog identiteta. Oslanjajući se na ove teorije, savremeni autori su celovitijem sagledavanju adolescencije pridodali odrednice: četrvtog organizatora psiha (6), drugog individualnog procesa (7), turbulentnog perioda sukcesivnog smenjivanja pubertetskog haosa, centralne narcističke depresije i ponovnog otkrivanja objekta (8), kao i definiciju „velike separacije" zbog čega dolazi do pojave anksioznosti različitog sadržaja i stepena izraženosti (9). Moglo bi se reći da je adolescencija prirodno data druga šansa da se sve nedorečenosti i sukobi dorade i reše. Ovako definisana prirodna mogućnost „dorade" često je praćena izraženim psihopatološkim sadržajima (10). Uobičajena ispoljavanja anksioznih i depresivnih kliničkih slika kod odraslih razlikuju se od manifestacije istih takvih psihopatoloških entiteta kod adolescenata. Adolescenti često strah i neraspoloženje ispoljavaju kroz bunt, a u zadnje vreme kroz specifičnu vitruelnu komunikaciju.

Paralelno, ali nezavisno, od pravilnog pozicioniranja adolescencije u psihijatrijskoj/psihološkoj literaturi, dolazi do razvoja socijalne psihijatrije sa stavljanjem akcenta na spoljašnje, sociološke faktore u razvoju psihopatologije. Definišu se pojmovi akcidentalne krize, traume i stresa, i njihov uticaj na svakodnevni život i razvoj psihopatologije. Dakle, kroz razvojne krize prolazimo svi, kroz akcidentalne samo oni koje nesreće, katasrofe i akcidenti zadese. Istovremeni prolazak kroz razvojnu i akcidentalnu krizu, uvek predstavlja potencijalnu opasnost.

U psihoterapijskom radu sa adolescentima i sporadičnim razgovorima sa njihovim roditeljima, koji su se obratili za savet i pomoć u vreme vanrednog stanja u Srbiji, izazvanog pandemijom Kovid-19, definisane su tri faze: prva faza predstavlja negaciju realne opasnosti, ispoljenu kroz zadovoljstvo zbog neodlaska u školu ili na fakultet; druga faza ispunjena je osećajem zbunjenosti i haosa zbog trajanja situacije u kojoj počinju da se mešaju doživljaji odmora (raspusta), obaveza i neizvesnosti, sa ispoljavanjem prvih, blažih, simptoma; treća faza prebojena je slomom odbrambenih mehanizama sa preplavljenošću strahom i depresijom, specifičnog načina ispoljavanja.

Cilj istraživanja je prikaz i analiza kliničkog psihoterapijskog materijala opisanih faza prolaska kroz akcidentalnu krizu, izazvanu pandemijom Kovid-19, kod adolescenata različitog uzrasta.

Metode

U radu su korišćeni materijali psihoterapijskih seansi adolescenata različith faza adolescentne krize. Za objašnjenje negacije realne opsnosti prikazani su adolescenti rane i središne faze adolescencije, a za osećaj zbunjenosti i haotičnosti do potpunog depresivnog sloma adolescenti kasne i postadolescentne faze.

Prikaz pacijenata i diskusija

Prva faza: negacija realnosti ispoljena kroz osećaj zadovoljstva i sreće zbog nemogućnosti odlaska u školu i ideje produženog raspusta bila je prisutna u velikom broju slučajeva u početku bez obzira na uzrast.

Tamara ima 12 godina, prvo je od dvoje dece četvoročlane porodice, koja živi u proširenoj porodici sa očevim roditeljima u velikoj porodičnoj kući. Uspešna je učenica VI razreda Osnovne škole i V razreda muzičke škole. Opisujući vanredno stanje kaže: „...meni kao da se ništa ne dešava, takav mi je osećaj, kao da sam na raspustu i da ove pandemije uopšte nema...". Ne prati sa lakoćom nastavu preko TV-a, ne shvata poentu toga, „...kao da je raspust...u školi sve zapamtim na času i kod kuće samo obnovim, a sad moram sve da učim...". Sa vršnjacima se ne čuje često, samo sa najboljom drugaricom. Za pomoć i savet obratili su se roditelji, jer je i za njihovu ćerku i za

of youth has been studied by historians and sociologists. Puberty changes have been studied from early ages in the sense of biological-physiological changes, mostly by pediatricians. Adolescence is the period, which is described by clinical psychotherapists: psychiatrists and psychologists (4,5). Freud's psychoanalytical thought offers the first explanation of adolescence, defining it as a transition period between childhood and adulthood. It is characterized by intense emotional tempests, frequent and sudden changes of mood and facing the roused Oedipal conflicts (5).

A psychiatrist-psychoanalyst E. Erikson paid more attention to adolescence in his theory of personality, according to which the whole life was divided into eight developmental normative crises, while adolescence was given the central place of the fifth normative crisis (1). It was emphasized once again that adolescence was the most tempestuous crisis in life, when all problems and good and bad solutions from previous life phases were faced, in order to synthesize all previous life experiences and build up a reliable and stable identity. By relying on these theories, contemporary authors added the following characteristics to a more comprehensive understanding of adolescence: the fourth organizer of psyche (6), the second individual process (7), the turbulent period of successive interchange of puberty chaos, central narcissistic depression and the repeated discovery of object (8), as well as the definition of "great separation", due to which anxiety appears, the anxiety of different content and level of expression (9). It could be said that adolescence is the natural second chance to solve and add the finishing touches to all the unsaid things and conflicts. This natural possibility of "adding the finishing touches" is often followed by the pronounced psychopathological contents (10). Usual manifestations of anxiety and depression in clinical practice among adults are different from manifestations of the same psychopathological entities in adolescents. Adolescents express fear and bad mood through disobedience, and lately through specific virtual communication.

In parallel, but independently the correct positioning of adolescence in psychiatric-psychological literature induces the development of social psychiatry with an emphasis on outer social factors in the development of psychopathology. The notions of accidental crisis, trauma and stress have been defined, as well as their influence on everyday life and the development of psychopathology. Therefore, all people undergo developmental crises, while accidental crises are experienced only by those people, who face misfortunes, catastrophes, accidents. The simultaneous experience of developmental and accidental crises always represents a potential danger.

Three phases have been defined during the psychotherapeutic work with adolescents and sporadic talks with their parents, who sought advice and help during the state of emergency in Serbia caused by the Covid-19 pandemic: the first phase represents the negation of real danger, which was expressed as satisfaction because they did not go to school or faculty; the second phase is filled with the feeling of confusion and chaos due to the duration of this situation, in which the sense of relaxation (holiday), obligations and uncertainty are mixed up when the first symptoms appear; the third phase is colored by the breakdown of defense mechanisms together with the overwhelming fear and depression, which are manifested in a specific way.

The aim of the research was to review and analyze the clinical psychotherapeutic material of the described phases through an accidental crisis, caused by the Covid-19 pandemic among adolescents belonging to different age groups.

Methods

The materials from psychotherapeutic sessions including adolescents in different phases of the adolescent crisis were used for the study. The adolescents of the early or central stages of adolescence were presented in order to explain the negation of real danger, whereas the adolescents of the late and post-adolescent phase were presented in order to explain the feelings of confusion and chaos to complete depressive breakdown.

Case reports and discussion

The first phase: the negation of danger expressed through the feeling of satisfaction and happiness because they could not go to

njih ova situacija zbunjujuća. Ne znaju kako da joj objasne da na „raspustu", mora da uči. Za sve obaveze u obe škole, koje je ranije sama obavljala, sada moraju da je opominju, organizuju i proveravaju…

Na samom početku adolescencije (prva faza), još nije počelo da se strukturiše apstraktno mišljenje. Problemi se ispoljavaju, prevashodno, kroz negiranje, zbunjenosti i prve nagoveštaje depresivnosti u vidu oslabljene koncentracije i usporenog odrađivanja školskih obaveza. Celokupna problematika opisana na konkretnom primeru je karakteristična za početnu fazu adolescencije i funkcionisanja u okviru konkretnog mišljenja. Bez obzira na malu razliku u godinama, ispostavilo se, i sada i u ranijim istraživanjima (11), da čak i osobe prvog razreda srednje škole, u odnosu na osnovnu školu, imaju kvalitativno bolji i strukturisaniji način razmišljanja i funkcionisanja.

Petar ima 15 godina, učenik je prvog razreda srednje stručne škole, prvo je od dvoje dece četvoročlane porodice. Porodica živi u stanu jedne centralne beogradske opštine. Oduševljenje zbog neodlaska u školu, vrlo brzo je zamenjeno osećajima „smorenosti", strepnje i poremećaja sna. Rekao je: „većina dece, kao i ja, mrzi da ide u školu! Prvih dana je bilo super, ali već u trećoj nedelji neodlaska u školu, ma koliko je ona dosadna, počelo je da mi smeta… navikao sam se da imam svoj termin i svoju rutinu… i smeta mi kad je izgubim…". Pomoć su potražili roditelji zbog dečakove uznemirenosti, preteranog ispoljavanja brige i nesanice. Petar je dve nedelje nakon početka vanrednog stanja počeo da verbalizuje intenzivnu brigu za mlađu sestru, roditelje, baku… Insistirao je na preciznom praktikovanju preporučenih higijenskih mera i na kraju je počeo da ispoljava probleme sa snom: dugo bi uveče ostajao budan, dok svi ne zaspu, ali se i tokom noći često budio i proveravao da li je sve u redu. Ujutru bi bio umoran i tokom dana rasejan i nervozan. Rekao je u razgovoru: „…vanredno stanje mi je pomoglo da shvatim koliko mi ljudi oko mene, u stvari, znače, koliko mi je tužno kad im se nešto desi… ova situacija jeste problematična, ali mi je pomogla da se zbližim sa onima koje volim…"

Usled centralne pozicije narcističke depresije u adolescentnom uzrastu, depresija često dobija drugi vid ispoljavanja. U ovom slučaju manifestovala se kroz altruistički oblik brige za druge. Dečaku je to dalo osećaj „zrelosti", koji je „popravio osećaj nemoći" i maskirao klasično ispoljavanje straha i depresije. Čak i kada govori o tuzi, on kaže: „*…tužno mi je kad im se nešto desi…*", bez korišćenja prvog lica jednine za osećaj tuge, koja se u njegovim godinama doživljava kao slabost. Ispoljiti strah i neraspoloženje na uobičajen način, neoprostiva je slabost za adolescente, zato je i prepoznajemo kroz haotičnost u smislu: „ *…na raspustu sam/ nisam na raspustu, učim/ne učim… ne znam šta sam i šta se dešava…*". U ovom trenutku mnogo je lakše reći „*…nedostaje mi škola iako je dosadna…*", nego priznati i verbalizovati strah od gubitka svakodnevnih rituala, koji imaju organizujuću funkciju.

Adolescenti koji su i pre pandemije imali ispoljenu i ozbiljniju psihopatologiju, brže su dospevali do druge faze u kojoj su dominirali osećaj zbunjenosti, haosa i ispoljavanje prvih, simptoma psihološke dekompenzacije. Ova dekompenzacija imala je skriveni, tj. somatskim problemima prepokriven sadržaj.

Hana je mlađe od dvoje dece četvorčlane porodice, visokog porodičnog materijalnog i socijalnog statusa. Ima 16 godina i pohađa I razred privatne škole. Dve godine unazad, jednonom nedeljno, dolazila je na pedesetominutnu individualnu psihoterapiju zbog ozbiljnih simptoma iz oblasti opseivno-kompulzivnog poremećaja i sklonosti somatizaciji. Situacija je zakomplikovana i postojanjem realnih somatskih poremećaja endokrinološkog sadržaja. Na početku pandemije, nastavila je da dolazi u ordinaciju žaleći se kako je taman smanjila broj pranja ruku, a sad je teraju da opet poveća učestalost tih rituala. Delovala je opušteno. Međutim, novi vid lečenja somatske problematike, zadesio se baš u vreme početka pandemije. Sam početak lečenja obeležen je ozbiljnim nuspojavama, za koje svetski priznati endokrinolozi nisu imali objašnjenje. Hana je skoro sasvim izgubila apetit i za kratko vreme smršala 5 kg. Taj gubitak je doveo do skoro anoreksičnog izgleda. Počela je da oseća vrtoglavice i nestabilnost pri hodu, te je skoro do krajnosti redukovala kretanje. Koncentracija za praćenje online nastave je drastično pala, a na kraju je i san postao problematičan. Nakon dvonedeljnog prekida, nastavila je psihoterpiju putem skajpa jednom nedeljno u trajanju od 50 minuta, kao do tada. Timksi je sagledana putem

school and because of the idea of a prolonged holiday was present in a great number of cases, regardless of age.

Tamara is twelve. She is the first of the two children in this family, which has four members. They live in a big family house together with her father's parents. She is a successful student in year six of Primary School and in year five of Music School. When describing the state of emergency, she says: "...it seems as nothing is going on, it's my feeling, as if I was on holiday, and as if there was no pandemic..." She does not watch classes on TV, she thinks it is meaningless, "... it's as if I was on holiday... when I go to school, I remember everything in class, and I just revise at home, and now I have to study everything..." She does not talk on the phone with her peers, only with her best friend. Her parents asked for advice and help because this situation has been confusing for them, and their daughter as well. They do not know how to explain to her that she must study "on holiday". She did everything on her own before, but now they have to remind her, organize and check...

At the very beginning of adolescence (the first phase), abstract thinking has not been structured yet. Problems appear, first of all, through negation, confusion and first signs of depression in the form of weak concentration and school duties are done with reduced speed. The first described example is characteristic of the beginning phase of adolescence and functioning within the concrete thinking. Although the age difference is small, it has been proven, now and in previous research (11) that even people in the first year of high school, in comparison to primary school, have a better quality and better structured way of thinking and functioning.

Petar is fifteen. He is in the first year of professional high school. He is the first of the two children, in the family that has four members. The family lives in a flat in a central Belgrade municipality. To his great delight he did not go to school, but soon this feeling was replaced by the feeling of boredom, apprehension, and sleep disorder. He said: "most of the kids hate going to school, and me too. At first it was great, but during the third week of not going to school, no matter how boring it is, it started to bother me... I got used to my routine, and it bothers me when I lose it..." Parents sought help because the boy was anxious, he worried too much and did not sleep well. Two weeks after the state of emergency had been introduced, Peter started to verbalize intense worry for his younger sister, parents, grandmother... He insisted on firm practicing of all recommended protective measures and in the end, he started to have sleep problems: he would stay up late at night, until everybody went to sleep, but he would rouse from sleep often and check if everything was all right. In the morning, he would be tired, and during day absent-minded and nervous. He said once: "... the state of emergency has helped me to realize how much people around me mean to me, how sad it is when something happens to them... this situation is problematic, but it brought together people I love..."

Due to the central position of narcissist depression in adolescence, depression often takes the other form of manifestation. In this case, it was manifested through the altruist form of worry for others. It gave this boy the feeling of "maturity", which fixed up the feeling of helplessness and masked the classical manifestation of fear and depression. Even when he speaks about sadness, he says: "*it's sad when something happens to them...*", without using the first person singular for the feeling of sadness, which is in his age seen as weakness. To show fear and bad mood in an unusual way is the unpardonable weakness for adolescents. Therefore, it is recognized as chaos: "*...I am on holiday/I am not on holiday, I study/I don't study... I don't know who I am and what is going on...*" At this moment, it is much easier to say: "I *miss school, although it is boring...*" than to admit and verbalize fear of losing everyday rituals, which have an organizing function.

Adolescents, who had had a more severely manifested psychopathology even before the pandemic, came more quickly to the second phase, in which the feelings of confusion, chaos and first symptoms of psychological decompensation were dominant. This decompensation had a hidden content that was overly covered with somatic problems.

Hana is the younger child of the two kids in the family of four members. The family is of high material and social status. She is 16 and she is in the first year of one private high school. During

online komunikacije i njoj i njenim roditeljima predložen je prekid medikamentozne terapije zbog somatske problematike. Hana je to odbila! Osim toga, nije bilo moguće ordinirati psihijatrijsku medikamentoznu terapiju, tako da je ostala samo mogućnost intenziviranja psihoterapije. Kroz tako intenziviran rad, uspela je sebi da približi psihološku pozadinu neobjašnjivih nuspojava nove somatske terapije. Suočavanje se odvijalo kroz dramatičan plač i negodovanje. Nakon toga, pokušala je da prekine samovoljnu kompletnu izolaciju i bar malo da se vrati kretanju. U početku je taj pokušaj zahtevao pomoć roditelja, što je dodatno urušavalo samopouzdanje.

Labilno i delimično uspostavljena ravnoteža normativne krize brzo se srušila, sa istovremenim proživljavanjem normativne i akcidentalne krize. Preplavljenost neprijatnim osećanjima dovela je do potpunog pogoršanja, ali i do čvrstog držanja za „uzročnika somatskih tegoba", tj. nove terapije. Ukoliko bi se odrekla nje, izgubila bi „objektivno" opravdanje za svoje somatske tegobe i morala bi da prizna svoju slabost (strah), što je za nju bilo teže prihvatljivo.

Adolescenti skloni bolestima zavisnosti, bilo da se radi o hemijskim ili nehemijskim zavisnicima, procenjeni su u ranijim istraživanjima kao najvulnerabilniji i najnedefinisaniji od svih psihopatoloških entiteta koje srećemo (5). Očekivalo se da će kod nekih od njih doći do pogoršanja.

Nikola ima 20 godina, student je prve godine fakulteta zdravstvene struke, živi sam, majka ga finansira. Imao je 10 godina kada su se roditelji razveli. Živeo je sa majkom, pa sa bakom (majčinom majkom), sa starijom sestrom i sada je sam. Već nekoliko godina unazad konzumira psihoaktivnu supstancu - kanabis, različite učestalosti konzumiranja, od svakodnevnog do jednom u deset dana, bez jasne pravilosti i razloga ove promenljive učestalosti konzumiranja. Imao je nekoliko ozbiljnih pokušaja lečenja. Jedan je od zagovornika postojanja lakih droga i zahteva legalizaciju istih. U periodu restrikcije kretanja, smanjena mu je mogućnost nabavke kanabisa, zbog čega je upotrebu istih morao da smanji. Njegova uznemirenost, bes i agresivnost, progresivno su se povećavali. Za razliku od prethodnih pacijenta, Nikola je verbalizovao povezanost svojih pogoršanja sa vanrednom situacijom, ali u smislu nemogućnosti nabavke kanabisa. Pokušao je sebe da smiri racionalnim objašnjenjem, ali bez uspeha. Intenziviranje medikamentozne terapije i savetodavnog rada donekle je ublažilo hetero i autoagresivnost kod njega.

Zavisnost od psihoaktivnih supustanci, uvek postaje poroblematična u kriznim situacijama, ali istovremeno pruža mogućnost „laganog" smanjivanja upotrebe istih, kod izvesnog broja adolescenata. Međutim, njihova neizdiferenciranost identiteta ostaje problem i psihoterapijski izazov i za adolescenta i za psihoterapeuta.

Zaključak

Superponiranje akcidentalne krize na normativnu razvojnu krizu uvek je klinički potencijalno opasno, ali i teorijski izazov za sagledavanje i definisanje novih obrazaca ponašanja i ispoljavanja psihopatologije. Pandemija Kovid-19, omogućila nam je da lakše sagledamo i definišemo faze prolaska adolecenata, različitog uzrasta, kroz superponiranu akcidentalnu krizu. To su jasno ispoljene tri faze: negiranje realne opasnosti; osećaj zbunjenosti i haosa sa početkom ispoljavanja prvih simptoma, koji dovode do laganog kompromitovanja novouspostavljenih obrazaca funkcionisanja i završnica u obliku depresivnog sloma, koji se manifestuje specifičnim ispoljavanjima: od zrelijeg pokušaja odbrane u vidu altruizma, preko nezrelije somatizacije, do ispoljavanja auto i heteroagresivnosti usled apsitinencijalnog sindroma. Iako klinička manifestacija ispoljene patologije deluje aktuelno ozbiljno, do nivoa dramatičnosti, može biti prolaznog karaktera. Svaka kriza, bilo da je razvojna, u ovom slučaju adolescentna, ili akcidentalna, u ovom slučaju pandemija Kovid-19, nosi u sebi veliki potencijal rasta i razvoja. Zbog psihoterapijskih intervencija, potencijal psihološkog rasta i razvoja u razvojnoj adolescentnoj krizi, može biti intenziviran. U krajnjem ishodu, uspešan izlazak iz superponirane normativne i akcidentalne krize, ovim može dovesti do ubrzanja izgradnje zrelijeg identiteta.

the last two years she used to come once in a week to individual psychotherapeutic sessions lasting fifty minutes due to severe symptoms connected with the obsessive-compulsive disorder and inclination to somatization. The situation was even more complicated due to the real somatic disorders of endocrine contents. At the beginning of the pandemic, she continued to come and she complained that just as she reduced washing hands, she was forced to increase the frequency of those rituals. She seemed relaxed. However, a new kind of treatment for somatic problems was introduced just at the beginning of pandemic. The very beginning of treatment was marked by serious side effects, which even the eminent endocrinologists could not explain. She lost appetite and lost almost 5 kg. This weight loss almost brought to an anorexic appearance. She started to feel dizziness and instability while walking and therefore she reduced movement to an extreme. She lacked concentration for online lectures, and in the end she had problems with sleep. After a two weeks interruption, she continued with psychotherapy via Skype once in a week and lasting 50 minutes, as before. The team examined her online and due to somatic problems the cessation of drug therapy was offered to her and her parents. Hana rejected it! Besides, it was not possible to practice the psychiatric drug therapy, and therefore, only the possibility of intensifying psychotherapy was left. The intensified work enabled her to understand better the psychological background of the inexplicable side effects of new somatic treatment. Confronting was going on with dramatic crying and protesting. Afterwards, she tried to stop the complete self-willed isolation and to move at least a little bit. At first, her parents had to help her, so it additionally ruined her self-confidence.

Unstable and partially established balance of normative crisis was soon destroyed with the simultaneous experience of normative and accidental crisis. Overwhelming feelings of unpleasantness brought to complete worsening, and to firm conforming to "causes of somatic problems", that is, the new treatment. If she denied it, she would lose "objective" justification for her somatic problems and she would have to admit her weakness (fear), which would be hard to accept.

Adolescents who are inclined to addictive disorders, no matter whether they abuse chemical or non-chemical substances, were assessed in the previous research as the most vulnerable and undefined among all psychopathological entities met in practice (5). Some of them were expected to have worsening of symptoms.

Nikola is twenty. He is the first-year student at one medical faculty. He lives alone and he is financed by his mother. He was ten when his parents divorced. He lived with his mother, and then with his grandmother (his mother's mother), with his older sister and now he is alone. He has used a psychoactive substance – cannabis for a few years, with a different frequency of consumption, from every day to once in ten days, without clear regularity and reason for this changeable frequency of consumption. He has had a few serious attempts of curing. He is one of the advocates of light drugs and he demands their legalization. During movement restrictions, the possibility of finding cannabis was smaller, and therefore, he had to reduce its consumption. His anxiety, rage and aggressiveness increased progressively. In contrast to previous patients, Nikola verbalized the connection of worsening of his state with the state of emergency, due to the problems with cannabis supply. He tried to calm himself down with some rational explanation, but without success. The intensification of drug therapy and counseling work relieved to some extent his hetero and auto-aggressiveness.

Dependence on psychoactive substances always becomes problematic in situations of crisis, but simultaneously it offers the possibility of a "slow" reduction of the consumption, among some adolescents. However, the undifferentiated identity has remained problematic and a psychotherapeutic challenge for an adolescent, and psychotherapist, as well.

Conclusion

Superimposing of an accidental crisis on the normative developmental crisis is always clinically potentially dangerous, but also a theoretical challenge for realizing and defining new patterns of behavior and manifestations of psychopathology. The Covid-19 pandemic made it possible for us to realize more easily and define

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the phases of going through superimposed accidental crisis in adolescents of different age. Three phases are clearly manifested: the negation of real danger; the feeling of confusion and chaos with the onset of first symptoms, which lead to slow compromising of newly established patterns of functioning and endings in the form of depressive breakdown, which has specific manifestations: from a more mature attempt of defense in the form of altruism to less mature somatization, and to auto and hetero-aggressiveness due to the abstinence syndrome. Although the clinical manifestation of the expressed pathology seems actual and serious to the extent of dramatics, it can be of transient character. Each crisis, whether developmental, in this case adolescent, or accidental, in this case caused by the Covid-19 virus, bears a big potential of development and growth. Due to psychotherapeutic interventions, the potential of psychological growth and development in the developmental adolescent crisis can be intensified. In the final outcome, a successful exit from the superponed normative and accidental crisis can thus lead to the quicker development of a more mature identity.

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DOSADAŠNJA SAZNANJA O ETIOPATOGENEZI I MOGUĆNOSTIMA TERAPIJE KOVID-19

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SAŽETAK

Trenutno je u svetu od Kovida-19 obolelo preko 35 miliona ljudi, a preko milion je umrlo. Pandemijski karakter bolesti je nametnuo dinamična istraživanja, kako u oblasti razjašnjavanja etiopatogenetskih mehanizama bolesti, tako i u oblasti moguće terapije. Količina naučnih znanja se u poslednjih devet meseci nekoliko desetina puta uvećala, ali i dalje nismo ni blizu definisanju efikasne i sigurne terapije. Saznanje da je Kovid-19 ne samo respiratorno, već i multisistemsko oboljenje, koje zahvata skoro sve organe, dalo nam je mogućnosti za terapijska prilagođavanja. Jak oksidacioni stres, tiha hronična hipoksija, povećana koagulabilnost i povećana agregacija trombocita, samo su neki od mehanizama u razvoju bolesti. Smatra se da su kardiovaskularne posledice i poremećaj funkcije endotela krvnih sudova uglavnom odgovorni za smrtni ishod, uz opasnu citokinsku oluju i akutni respiratorni distres sindrom. Imajući sve ovo u vidu, u terapijskom smislu, kod već obolelih osoba, predložena je primena antibiotika, visokih doza vitamina C zbog antioksidacionog dejstva, transfuzije krvi, imunoglobulina, interferona, anti-IL-6 antitela, malih doza deksametazona ili drugih kortikosteroida, specifičnih antivirusnih lekova, favipiravira i remdesivira. Primena hlorokina isključena je iz terapijskih protokola Svetske zdravstvene organizacije i američkih Centara za kontrolu i prevenciju bolesti. U preventivne i suportivne svrhe savetuju se visoke doze vitamina D, vitamina C, cinka, probiotika, alfalipoinske kiseline i drugih suplemenata. Primena antinflamatornih analgo-antipiretika smatra se opravdanom, kao i primena malih doza acetilsalicilne kiseline. Preko 70 drugih lekova se trenutno ispituje u preko 400 kliničih studija. Kod pacijenata koji već inhalatorno ili intranazalno koriste kortikosteroide zbog astme, hronične opstruktivne bolesti pluća, alergijskog rinitisa, ili biološke lekove zbog imunomodulisanih inflamatornih bolesti, ovu terapiju ne treba menjati i ona nije preduslov za teže oblike bolesti. Moraju se uzeti u obzir i specifičnosti infekcije kod posebnih populacija kakve su deca ili trudnice.

Ključne reči: Kovid-19, etiopatogeneza, terapija

Uvod

Pandemija izazvana novim korona virusom (SARS-CoV-2) pogodila je preko 35 miliona ljudi širom sveta i odnela preko milion ljudskih života. I dok se svet borio sa epidemiološkim problemima i preventivnim merama, dok su se lekari po bolnicima borili za živote ljudi sa teškim oblikom Kovid-19, grupe i pojedinci u svetu nauke i struke pokušavali su da nađu odgovore na brojna pitanja vezana za etiopatogenezu ove bolesti i za terapijske mogućnosti. Nikada u istoriji nauke nije bilo perioda kada je pojavljivanje novih i preispitivanje starih naučnih činjenica imalo ovakvu dinamiku kao u poslednjih 8 ili 9 meseci. I dalje ne znamo mnogo, nemamo specifični antivirusni lek, kao ni klinički verifikovanu i odobrenu vakcinu.

Cilj ovog preglednog rada je da prikaže dosadašnja znanja vezana za etiopatogenezu i terapiju Kovid-19.

Etiopatogeneza Kovida-19

Čini se da je veliki korak ka boljem lečenju i manjem umiranju učinjen kada smo shvatili da Kovid-19 nije samo respiratorno, već multisistemsko oboljenje. Moglo bi se reći da je to pre svega hematološko i kardiovaskularno oboljenje. Izgleda da promene koje se javljaju na plućima, i koje daju karakterističnu sliku na snimku, nisu posledica prave pneumonije, nego pre mikro plućne tromboze, prevashodno venskih krvnih sudova. Mikrotromboza se izgleda dešava i u drugim delovima tela (1).

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CURRENT KNOWLEDGE ABOUT THE ETIOPATHOGENESIS AND THERAPY OPTIONS FOR COVID-19

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SUMMARY

Currently, over 35 million people in the world are infected with the COVID-19 and over a million have died. The pandemic character of the disease has imposed dynamic research both in the field of clarification of the etiopathogenetic mechanisms of the disease and in the field of possible therapy. The amount of scientific knowledge has increased dozens of times in the last nine months, but we are still not even close to define an effective and safe therapy. The knowledge that COVID-19 is not only a respiratory but also a multisystem disease, which affects almost all organs, gave us opportunities for therapeutic adjustments. Strong oxidative stress, silent chronic hypoxia, increased coagulability, and increased platelet aggregation are just some of the mechanisms in the development of the disease. Cardiovascular consequences and vascular endothelial dysfunction are thought to be mainly responsible for death with a dangerous cytokine storm and acute respiratory distress syndrome. Having all this in mind in the therapeutic sense, it is proposed to use antibiotics, high doses of vitamin C, blood transfusion, immunoglobulin, interferon, anti-IL-6 antibodies, small doses of dexamethasone or other corticosteroids, specific antiviral drugs such as favipiravir and remdesivir. The use of chloroquine is excluded from the therapeutic protocols of the World Health Organization and the Centers for Disease Control and Prevention (United States of America). For preventive and supportive purposes, high doses of vitamin D, vitamin C, zinc, probiotics, alpha-lipoic acid, and other supplements are recommended. The use of anti-inflammatory, analgo-antipyretics drugs is considered justified, as well as the use of small doses of acetylsalicylic acid. Over 70 other drugs are currently being tested in over 400 clinical studies. In patients who already use corticosteroids by inhalation or intranasally due to asthma, chronic obstructive pulmonary disease, allergic rhinitis, or biological drugs due to immunomodulatory inflammatory diseases, this therapy should not be changed and it is not a prerequisite condition for more severe forms of the disease. The specifics of the infection in special populations such as children or pregnant women must also be taken into consideration.

Key words: COVID-19, etiopathogenesis, therapy

Introduction

The pandemic caused by the novel corona virus (SARS-CoV-2) has affected over 35 million people around the world and taken more than million lives. While the world struggled with epidemiological problems and preventive measures, and doctors fought against the severe forms of Covid-19 infection in hospitals, some groups and individuals from the scientific and professional world tried to find answers to numerous questions connected with the etiopathogenesis of this disease and possible therapy. There have been no periods in the history of science when the appearance of new and re-examination of old scientific facts had such dynamics as during the last 8 or 9 months. We still do not know much, we do not have a specific antiviral medicine, or clinically verified and approved vaccine.

The aim of this review article was to present the current knowledge about the etiopathogenesis and therapy of Covid-19.

The etiopathogenesis of Covid-19 infection

It seemed that a big step was made towards better treatment and smaller mortality when we realized that Covid-19 infection is not only a respiratory, but a multisystem disease, as well.

Obdukcioni nalaz nemačkih patologa objavljen u avgustu 2020. godine pokazao je da pacijenti inficirani SARS-CoV-2 virusom umiru uglavnom od koagulopatija, poremećaja funkcije endotela krvnih sudova ili poremećaja funkcije ćelija srčanog mišića. Virus utiče na funkciju endotela, on gubi svoja antiadhezivna, antiagregaciona i antiinflamatorna svojstva, manje se luči azot monoksid (NO) i nastaje *endotelitis* praćen povećanom sintezom protrombotičnih alfa defenzin proteina, što je najčešće glavni uzrok smrtnih ishoda kod Kovid-19 bolesti. Ovo je i razlog promena na bubrezima i drugim vitalnim organima i njihovog akutnog otkazivanja (2).

Sa druge strane, virus se svojim „*spike*" proteinima vezuje za angiotenzin-konvertujući enzim 2 (ACE2) receptore u epitelu respiratornog trakta, ali i drugim delovima tela, i ulazi u ćelije. Ekspresija ovih receptora je najveća u nosnoj sluzokoži i opada ka donjim partijama respiratornog sistema, te se nos smatra glavnim mestom ulaska visrusa u naše telo (3). Osim pluća, virus na ovakav način ulazi i u druge ćelije, ali se naročito važnim smatra način na koji on napada eritrocite. Postavljena je hipoteza da se virus zahvaljujući specifičnim proteinima vezuje za beta lanac hemoglobina i iz njega istiskuje gvožđe (Fe) (4). Posledica toga je visok nivo slobodnog Fe^{2+} u krvi, kada ono ima toksičnu ulogu, i svojim snažnim oksidativnim kapacitetom dovodi do sistemskog oksidativnog stresa i oštećenja skoro svih organa. Sa druge strane, hemoglobin nema vezano gvožđe koje bi prenosilo kiseonik, pa nastaje tiha hronična hipoksija sa posledičnim hipoksičnim oštećenjima čitavog organizma. Druga važna komplikacija Kovid-19 bolesti se objašnjava hiperimunim odgovorom, tzv. citokinska oluja, koju karakteriše povišen nivo i hiperaktivnost primarno interleukina 6 (IL-6), interleukina 1b (IL-1b) i faktor nekroze tumora alfa (TNF-alfa) i citokina (5,6), zbog čega sam imunski odgovor obolele osobe vrši destrukciju plućnog tkiva, a nagomilane i aktivirane ćelije imunskog sistema dodatno oslobađaju inflamatorne medijatore i oštećuju krvne sudove, što se dešava u najtežim kliničkim slučajevima akutnog respiratornog distres sindroma (5,6).

Teorijski gledano, ovakav patogenetski mehanizam zahteva da pacijent dobije transfuziju sveže krvi (nadokanada kiseonika), visoke doze vitamina C intravenski (1,5 do 2 g dnevno) kao antioksidansa, te antikoagulanse (prednost se daje niskomolekularnom heparinu). Visoke doze vitamina D se daju pacijentima starije životne dobi sa utvrđenim deficitom, a cilj je da nivo 25-hidroksi vitamina D bude u okviru referntni vrednosti (40-60 ng/ml) (7).

Imajući u vidu da pacijenti sa hipertenzijom, dijabetesom ili oni koji su gojazni imaju već bazalno nizak nivo NO, smatra se da je to razlog njihove povećane sklonosti ka obolevanju i težim oblicima Kovid-19 bolesti (8). Moguća inhalatorna primena NO ili tadalafila (inhibitora fosfodiesteraze tipa V), u manjim dozama, može predstavljati terapisku opciju u budućnosti (9).

Kod pacijenta u biohemijskim analizama dominira visoko serumsko gvožđe, feritin, transaminaze, laktat dehidrogenaza, monociti, limfopenija, trombocitopenija i povišen nivo D dimera.

Terapijske mogućnosti u lečenju Kovida-19

Kako se donekle rasvetljavala etiopatogeneza, tako se i nametala moguća terapija. U početku nije bilo nikakve terapije koja bi se mogla smatrati specifičnom. Bilo je pokušaja sa preparatima interferona alfa u obliku nebulizatora ili spreja kod dece sa relativno slabim terapijskim uspehom. Prva antivirusna terapija koja se koristila bio je anti-influenca lek oseltamivir, koji skoro da nije dao nikakve rezultate, kao ni antivirusni lek za HIV ribavirin. Neki početni uspesi su se javili posle primene fiksne anti-HIV kombinacije lopinavir/ritonavir. Ubrzo se posle početka primene pojavila studija koja je pokazala da ovaj lek nema željeni terapijski uspeh te je napušten, osim kod trudnica gde se drugi antivirusni lekovi ne mogu davati (10).

Hlorokin u lečenju Kovida-19

Prvi lek koji nam je dao nadu u stvari je stari antimalarik hlorokin i njegov sintetski derivat hidroksihlorokin. Ovi lekovi su dugo u upotrebi, ali kao antiinflamatorni lekovi kod pacijenata sa sistemskim lupusom ili reumatoidnim artritisom, kao i u terapiji malarije. Od prvih terapijskih protokola u Vuhanu, do nesmotrene izjave američkog predsednika Trampa da se radi o, takoreći, čudotvornom leku, preko studije

It could be said that it is, first of all, hematologic and cardiovascular disease. It seems that the changes that appear on the lungs and which give a characteristic picture on an X-ray are not just a consequence of real pneumonia, but more of micro pulmonary thrombosis, primarily of venous blood vessels. Micro thrombosis appears in other parts of the body, as well (1).

The autopsy finding of German pathologists, which was published in August 2020, showed that patients infected with SARS-CoV-2 virus died mainly of coagulopathy, endothelial dysfunctions of blood vessels or heart muscle cell dysfunction. The virus affects endothelial function, and it loses its anti-adhesive, anti-aggregation and anti-inflammatory characteristics, the secretion of Nitric Oxide is smaller and endotheliitis appears followed by the increased synthesis of prothrombin alpha defensin proteins, which is most frequently the main cause of deadly disorders in Covid-19 infection. This is the reason of changes that appear on kidneys and other vital organs and their acute failure (2).

On the other hand, the virus with its "spike" proteins creates a bond with angiotensin, converting the enzyme 2 (ACE2) receptors in the epithelium of respiratory tract, as well as in other body parts, and it enters the cells. The expression of these receptors is highest in the nasal mucosa and it decreases towards the lower parts of respiratory tract, and therefore, nose is deemed to be the main place of the entry of this virus into our body (3). In addition to lungs, the virus enters in this way into other cells, but what is of great importance is the way in which it attacks erythrocytes. It has been hypothesized that the virus binds to the beta chain of hemoglobin thanks to specific proteins and displaces iron from it (4). The consequence of that is the high level of "free" iron, when it becomes toxic, and with its oxidative capacity it leads to the systemic oxidative stress and damage of almost all organs. On the other hand, hemoglobin does not have bound Fe which would transfer oxygen, and therefore quiet chronic hypoxia appears with the consequent hypoxic damages of the whole organism. The other important complication of Covid-19 is explained by the hyper-immune response, the so-called cytokine storm, which is characterized by the increased level and hyperactivity primarily of interleukin 6 (II-6), interleukin 1b (IL-1b) and tumor-necrotizing factor alpha (TNF-a) and cytokine (5,6), due to which the immune response of the patient destroys the lung tissue, and accumulated and activated cells of the immune system additionally liberate inflammatory mediators and damage blood vessels, which happens in the most difficult clinical cases of acute respiratory distress syndrome (5,6).

Theoretically speaking, such pathogenetic mechanism demands that the patient gets transfusion of fresh blood (compensation of oxygen), high doses of vitamin C intravenously (1.5 to 2 g daily) as the antioxidant, and then anticoagulants (low molecular weight heparin is preferred). High doses of vitamin D are given to elderly patients with established deficiency and the goal is to keep the level of 25-hydroxy vitamin D within the reference values (40-60 ng/ml) (7). Having in mind that patients with hypertension, diabetes and obese patients have a low level of basal NO, it is considered to be the reason of their increased tendency to get the disease and more severe forms of Covid-19 (8). A possible application of NO and tadalafil in inhalation (inhibitors of phosphodiesterase type V) in smaller doses, can present a therapy option in the future (9).

In patients, high serum iron, ferritin, transaminase, lactate dehydrogenase, monocytes, lymphopenia, thrombocytopenia and increased level of D dimer are dominant in biochemical analyses.

Therapy options for Covid-19

As the etiopathogenesis was slowly cleared up, the possible therapy was accepted. At the beginning there was no specific therapy. There were attempts to use the preparation of interferon in the form of nebulizers or spray in children with relatively weak therapeutic success. The first antiviral therapy was the anti-influenza medicine oseltamivir, which did not give any results, as well as the antiviral medicine for AIDS ribavirin. Some success was achieved after the administration of fixed anti AIDS combination of lopinavir/ritonavir. Soon after the beginning of administration, a study appeared which showed that this medicine

marsejskih virusologa sa skoro 100% uspehom u kombinaciji sa azitromicinom, do otrežnjenja vezanog za njegova neželjena dejstva i predloga francuskog ministra zdravlja da se ovaj lek više ne daje. Ono što nam nedostaje su dobro planirane randomizovane kliničke studije koje bi pokazale njegovu delotvornost i sigurnost. Ovaj stari lek ima mnogobrojne mehanizme dejstva, on sprečava da virus istisne gvožđe iz hemoglobina, sprečava ulazak virusa u ćelije domaćina, njihovu replikaciju i izlazak iz ćelija, smanjuje sintezu proinflamatornih citokina važnih u citokinskoj oluji, pre svih IL-6 i TNF-alfa i deluje proimunski (11,12). Po ranijim verzijama nacionalnog protokola bilo je predloženo da se hidroksihlorokin daje na sledeći način: tbl. 2 × 400 mg p.o. prvi dan (2 × 600 mg ukoliko pacijent ima nazogastričnu sondu), zatim 400 mg p.o. još 7 dana, u zavisnosti od kliničke slike. Pedijatrijske doze su: početna doza 2 × 6.5 mg/kg (maksimalno 400 mg) prvi dan, zatim 2 × 3.5 mg/kg na dan (maksimalno 200 mg) 4 dana. Osim toga, postoji velika mogućnost da ovaj lek izazove srčane aritmije, naročito kod predisponiranih pacijenata sa komorbiditetima, pa je od početne euforije došlo do velikog otrežnjenja da se lek mora davati samo uz velike mere predostrožnosti i posebnu pažnju (13). Na osnovu mnogobrojnih studija koje su pokazale neefikasnost hlorokina (14), Svetska zdravstvena organizacija (SZO) je predložila da se dalje ne daju dozvole za kliničke studije sa njim, Agencija za hranu i lekove (FDA) je to podržala, a mnoge zemlje su ga izbacile iz svojih protokola uključujući i Srbiju. Sa druge strane, grupe istraživača i dalje veruju i pokušavaju da daju dokaze da hidroksihlorokin sam, ili u kombinaciji sa azitromicinom, naročito ako se pravovremeno primeni, i dalje predstavlja jedan od ključnih lekova u terapiji Kovid-19. Da li je hidroksihlorokin zaista bezvredan, ili je žrtva igara i intriga velikih igrača na farmaceutskom svetskom tržištu, vreme će pokazati (15-17).

Antivirusni lekovi u lečenju Kovida-19

Osim neuspelih antivirusnih lekova, bilo je pokušaja sa kamostat mesilatom i umifenovirom, ruskim antivirusnim lekom, koji su u početku dosta obećavali. Rezultat je i ovde bio polovičan. Ovi lekovi deluju tako što se zakače za „*spike*" proteine virusa i spreče njihov kontakt sa ACE2 receptorima i ulaz u ćeliju (9). Danas se intezivno klinički ispituju 2 antivirusna leka, koja su već i registrovana u nekim zemljama. Oba su bila inovirana u prethodnim SARS, MERS i ebola infekcijama, ali njihova klinička istraživanja nisu bila privedena kraju. Favipiravir je u mnogim zemljama u III fazi kliničkih ispitivanja, a FDA je već dala zeleno svetlo za ubrzanu registraciju remdesivira, za kojeg se očekuje da će biti specifični antivirusni lek kod odraslih. Favipiravir je registrovan u Rusiji, Kini i Indiji, ali se koristi i ima ga u skoro svim nacionalnim protokolima. Može se davati oralno, od rane faze i već kod blagih oblika bolesti (tablete 1.600 mg na 12 h, prvi dan, od 2 - 5. dana (tj. još 4 dana) 600 mg na 12 h, ukupno 5 dana) (18).

Drugi antivirusni lek, remdesivir, je odobren u Sjedinjenim Američkim Državama (SAD), za lečenje teških oblika bolesti, kod kojih je potreban kiseonik, kod odraslih i dece starije od 12 godina. Vlada SAD i predsednik Tramp, su međutim otkupili godišnju proizvodnju ovog leka od proizvođača i time je ostatak sveta ostao uskraćen. Kliničke studije su pokazale efikasnost, manju učestalost teških kliničkih slučajeva, brže ozdravljenje i ublažavanje simptoma (200 mg prvi dan i.v., pa 100 mg i.v. sledećih 9 dana, po nalogu infektologa) (19).

Antiinflamatorni lekovi u lečenju Kovida-19

Od primene interferona (alfa-2b i beta-1b) bilo je pokušaja da se ova bolest leči imunološkim preparatima. Najviše dobrih rezultata je bilo sa tocilizumabom (8 mg/kg i.v. u dve doze, a maksimalno 800 mg po dozi), monoklonskim antitelima protiv IL-6 receptora, kao i sarilumabom ili siltuksimabom koji se koriste u reumatološkim inflamatornim oboljenjima. Završene su studije koje dovode u sumnju efikasnost tocilizumaba. Ovi lekovi ostaju terapijska opcija, naročito u teškim oblicima bolesti (20). Intravenski imunoglobulini u dozi 10 do 20 mg/kg u toku 3 do 5 dana su takođe terapijska opcija (21).

Plazma pacijenata koji su preležali bolest je terapija koja dosta obećava, pitanje je, međutim, u kojoj meri je ona bogata specifičnim antitelima koja mogu pomoći obolelima od Kovid-19. Nedavno je u SAD data dozvola da se plazma rekonvalescenata uvrsti u regularnu terapiju pacijenata sa Kovid-19 (22). Na osnovu

did not have a desired therapeutic success and therefore, it was left, except in pregnant patients, who could not be given other antiviral medicines (10).

Chloroquine in the treatment of Covid-19

The first medicine, which gave us hope, was actually an old antimalarial chloroquine and its synthetic derivative hydroxychloroquine. These medicines have been used for a long time, but as anti-inflammatory medications in patients with systemic lupus or rheumatoid arthritis as well as in malaria therapy. Since the first therapeutic protocols in Wuhan, to the incautious statement of the American president Trump that it was a miraculous drug, and the study of virologists from Marseille that it was 100% successful in combination with azithromycin, as well as to the sobering effect connected with its side effects and the proposal of the French Minister of Health not to give this medicine anymore. Well-planned randomized clinical studies, which would show its effectiveness and security, are still missing. This old medicine has numerous mechanisms of action; it prevents the virus from displacing iron from the hemoglobin, it prevents the entry of the virus into the cells of the host, its replication and exit from the cells, it diminishes the synthesis of pro-inflammatory cytokines, which are important in the cytokine storm, first of all, all interleukins IL-6 and TNF-alpha and it has a pro-immune effect (11,12). According to earlier versions of the national protocol, it was suggested that hydroxychloroquine should be given in the following way: pills 2 x 400 mg p.o. during the first day (2 × 600 mg if the patient has a nasogastric tube), then 400 mg p.o. seven days more, depending on the clinical picture. Pediatric doses are: the starting dose 2 × 6.5 mg/kg (max 400 mg) on the first day, then 2 × 3.5 mg/kg daily (max 200 mg) for four days. In addition to that, there is a great possibility that this medicine causes cardiac arrhythmia, especially in predisposed patients with comorbidities, and therefore, the starting euphoria turned into great sobriety which meant that this medicine could be given only with great measures of precaution and with special attention (13). According to numerous studies, which showed the inefficiency of chloroquine (14), the World Health Organization suggested that clinical studies with this medicine should not be approved, and the Food and Drug Administration supported this, while a lot of countries have removed it from their protocols, including Serbia, as well. On the other hand, some groups of researchers still believe and try to find proofs that hydroxychloroquine alone or in combination with azithromycin, especially if it is administered on time, still presents one of the key drugs for the treatment of Covid-19 infection. The time will show whether hydroxychloroquine is really worthless or it is the victim of intrigues and games of big players in the pharmaceutical world market (15, 16,17).

Antiviral drugs in the treatment of Covid-19 infection

In addition to the unsuccessful antiviral drugs, there were attempts to use camostat mesylate and umifenovir, a Russian antiviral drug, and they were promising in the beginning. The result was partial in this case, as well. These drugs bind to the "spike" proteins of the virus and thus prevent their contact with ACE2 receptors and the entry into the cell (9). Today, two antiviral drugs are being examined intensively, and they have already been registered in some countries. Both drugs were innovated in the previous SARS, MERS and Ebola infections, but their clinical research was not brought to an end. Favipriavir is in many countries in phase III of clinical research, while the FDA has already given a green light for the faster registration of remdesivir, which is expected to be a specific antiviral drug for adults. Favipiravir has been registered in Russia, China and India, but it is used and it is present in almost all national protocols. It can be administered orally, from the early phase and in mild forms of disease (tablets 1600 mg per 12 hours, during the first day, and from the 2nd to the 5th day (that is, four days more) 600 mg per 12 hours, in total for five days) (18).

The other antiviral drug, remdesivir got a green light in the United States of America for the treatment of severe forms of disease in adults and children older than 12. The Government of the United States of America and the president Trump purchased the production

verzije 9 našeg terapijskog protokola, plazma rekonvalescenata se može uvesti u terapiju unutar 2 nedelje od početka tegoba i nakon konsultacije sa infektologom i transfuziologom.

Antiinflamatorna terapija je nedvosmisleno potrebna. U početku pandemije WHO i druge organizacije imale su stav da je lek izbora paracetamol (do 1.500 mg na dan) jer nesteroidni antiinflamatorni lekovi (NSAIL) navodno potenciraju teže oblike bolesti. Ubrzo su naučne studije i stavovi mnogih državnih i stručnih organizacija razbile ovaj mit, pa se danas NSAIL upotrebljavaju paralelno sa paracetamolom (ibuprofen do 1.200 mg na dan). Neki eksperti skloni su preporuci za upotrebu malih doza acetilsalicilne kiseline zbog antiagregacionog dejstva (23). Ne zna se da li ovo može prevenirati mikrotromboze.

Primena sistemskih kortikosteroida se inače smatra racionalnom samo kod najtežih slučajeva i samo pod određenim uslovima. Oni se inače smatraju imunosupresorima i agensima koji povećavaju replikaciju virusa. Neki autori su skloni da preporuče metilprednizolon (1-2 mg/kg, 3 do 5 dana uz nadzor intenziviste), neki prednizon (0,5 mg/kg u 2 dnevne doze, pri čemu večernja doza iznosi maksimalno 10 mg, trajanje terapije je 4 nedelje, a doze prednizona se smanjuju po šemi), a neki hidrokortizon. Kliničke studije sa malim dozama deksametazona (6 mg/dan, deset dana) kod pacijenata na mehaničkoj ventilaciji ili kiseoničnoj potpori su pokazale dobre rezultate, smanjenje letaliteta i poboljšanje kliničke slike, te se tako deksametazon nameće kao vodeći kortikosteroid u terapiji Kovid-19 oboljenja (24). Prema našem protokolu (verzija 9) predlaže se primena metilprednizolona 1-2 mg/kg, 3-5 dana (uz procenu rizika i saglasnost intenziviste/infektologa/pulmologa).

Ukoliko su pacijenti sa imunološki posredovanim inflamatornim bolestima na kortikosteroidima i obole od Kovid-19 preporučuje se njihovo ukidanje, ako je moguće održati remisiju bez njih, ili prebacivanje na budesonid za koji se smatra da ima najmanji imunosupresivni potencijal. Biološku terapiju kod ovih pacijenata ne treba prekidati, jer ona ne potencira sklonost ka težim oblicima bolesti. Naprotiv, inhibitori TNF-alfa čak mogu poboljšati simptome bolesti, jer se smatra da je on pokretač citokinskog odgovora, te se neki od njih i ispituju kao mogući antikovid agensi (25).

Mnogobrojne organizacije koje se bave alergijskim bolestima, astmom ili hroničnom opstruktivnom bolešću pluća (EUFOREA, ARIA, GINA, GOLD, EACI i dr.) su zauzele stav da intranazalne i inhalatorne kortikosteroide ne treba prekidati ukoliko ovi pacijenti dobiju Kovid-19. Naprotiv, postoji stav da lokalni kortikosteroidi smanjuju ekspresiju ACE2 receptora i replikaciju virusa, a da je njihova sistemska resorpcija mala, te mogu da imaju i olakšavajuću funkciju ili čak i preventivnu (26).

Američki lekar Ričard Bartled je čak predložio terapijski koktel: inhalatorni budesonid, cink, klaritromicin i acetilsalicilna kiselina, kao bazični set lekova za lečenje Kovid-19 oboljenja već u ranoj fazi bolesti. Moguća primena inhalatornog budesonida u prevenciji prelaska u teže oblike bolesti i u bržem ozdravljenju delimično je potvrđena i rezultatima STOIC studije. Uprkos tome neke druge grupe istraživača osporavaju ovakav koncept (27).

Postoji još jedna procedura, koja predstavlja hemodijalizu kojom se iz tela odstranjuju citokini u toku citokinske oluje. Metoda se zove Citosorb, ali je, nažalost, skupa i nije pogodna za masovnu upotrebu (28).

Antibiotska terapija

Primena antibiotika smatra se neophodnom kod ARDS-a ili prema antibiogramu ili kod lečenja već nastale sekundarne bakterijske infekcije.

Moguće perspektive u lečenju Kovida-19

Nikada u svetu farmakoloških istraživanja nije bilo ovakve dinamike u naučnim i stručnim pokušajima i ovoliko mnogo istraživanja. Informacija stiže informaciju i trenutno se u svetu istražuje najmanje 70 starih ili novih lekova sa mogućnošću primene kod Kovid-19 infekcije. Najmanje je 400 kliničkih studija u toku. I dalje smo terapijski u potpunom mraku, sa malim, ali nedovoljnim pomacima, međutim, očekujemo da se ubrzo pojave vakcine i da imamo specifičnu antivirusnu terapiju.

Prevencija Kovida-19

Suplementacija i moguća preventiva kod ove bolesti su predmet stalnog intersovanja.

of this drug from the manufacturer for year and thus deprived the rest of the world of it. Clinical studies showed efficacy, lower incidence of severe clinical cases, faster recovery and relief of symptoms (200 mg during the first day iv, and then 100 mg iv during the following 9 days, by the order of infectologist) (19).

Anti-inflammatory drugs in the treatment of Covid-19

Since the administration of interferon (alfa-2b and beta-1b), there have been attempts to treat this disease with immunological preparations. Good results were achieved mostly by tocilizumab (8 mg/kg i.v. in two doses, and maximally 800 mg per dose), monoclonal antibodies against IL-6 receptors, as well as by sarilumab or siltuximab, which are used for rheumatologic inflammatory diseases. These drugs have remained the therapeutic option, especially in severe forms of disease (20). Intravenous immunoglobulins in doses 10 to 20 mg/kg during 3 to 5 days are also a therapeutic option (21).

The plasma of patients, who have overcome this disease, is still promising. The question remains to what extent it is abundant in specific antibodies, which could help in curing people with Covid-19. An approval has recently been made in the United States of America to include the plasma of convalescents in the regular therapy of patients with Covid-19 (22).

The anti-inflammatory therapy is unambiguously necessary. At the beginning of pandemic, the position of the WHO and other organizations was that paracetamol was the drug of choice (to 1.500 mg a day) because the non-steroidal anti-inflammatory drugs (NSAIL) allegedly induce more severe forms of disease. Soon the scientific studies and positions of many state and professional organizations dispersed this myth, and today NSAIL are used in parallel with paracetamol (ibuprofen to 1.200 mg daily). Some experts tend to accept the recommendation to use small doses of acetylsalicylic acid due to the anti-aggregation effect (23). It is not known whether this can prevent micro-thrombosis.

The administration of systemic corticosteroids is deemed to be rational only in most severe cases and under certain circumstances. They are deemed to be immunosuppressive drugs and agents that increase the virus replication. Some authors tend to recommend methylprednisolone (1-2 mg/kg, 3 to 5 days under the supervision of intensivist), some recommend prednisone (0.5 mg/kg I 2 daily doses, while the evening dose amounts to max. 10 mg, and duration of therapy is 4 weeks, and doses of prednisone are reduced according to the plan), while some recommend hydrocortisone. Clinical studies with small doses of dexamethasone (6 mg/a day, 10 days) in patients on mechanical ventilation or oxygen support have showed good results, reduced mortality and improved the clinical picture, and therefore, dexamethasone is imposed as a leading corticosteroid in the treatment of Covid-19 infection (24).

If patients with immunologically mediated inflammatory diseases on corticosteroids get infected with Covid-19, their usage is recommended to be cancelled if remission is possible to be maintained without them, as well as transition to budesonide which has the least immunosuppressive potential. Biological therapy in these patients should not be stopped, because it does not induce the inclination to more severe forms of disease. On the contrary, inhibitors TNF-alpha can even improve the symptoms of disease, because it is deemed to be the initiator of cytokine response, and therefore, some of them are examined as possible anti-Covid agents (25).

Numerous organizations, which deal with allergic diseases, asthma or chronic obstructive lung disease (EUFOREA, ARIA, GINA, GOLD, EACI etc.), have taken the position that patients who get Covid-19 should not stop to use intranasal and inhaled corticosteroids. On the contrary, there is opinion that local corticosteroids reduce the expression of ACE2 receptors and the virus replication, and that their systemic reabsorption is small, and therefore, they can have a relieving function or even a preventive one (26).

An American doctor, Richard Bartlett even suggested a therapy cocktail: inhaled budesonide, zinc, clarithromycin and acetylsalicylic acid, as a basic set of drugs for treating Covid-19 infection in the early phase of disease. The possible administration of inhaled budesonide in the prevention of transition to more severe forms of disease and in faster recovery has partially been

Nesporna je preventivna uloga visokih doza vitamina D (10.000 IJ na dan u toku nekoliko nedelja, posle čega se može preći na 5.000 IJ na dan) (29). Vitamin D ima svoje receptore i na ćelijama urođenog i na ćelijama stečenog imuniteta, pospešuje stvaranje antimikrobnih protina, ublažava citokinsku oluju i sintezu proinflamatornih citokina, ublažava sva stanja koja kao komorbiditeti pogoršavaju Kovid-19 i ima mnogobrojne druge terapijske efekte.

Zbog smanjenja proinflamatornih uticaja i subepitelne lokalne metaboličke inflamacije u crevima koja je preduslov za sistemsku inflamaciju, probiotici se smatraju racionalnom preventivom i terapijom (30).

Cink (najmanje 15 mg dnevno) očigledno sprečava replikaciju virusa, polinezasićene masne kiseline smanjuju sintezu proinflamatornih supstanci, vitamin C (500 mg) deluje antioksidanto i imunomodulatorno, alfalipoinska kiselina (300 do 600 mg) deluje neuroprotektivno i popravlja i prevenira anosmiju.

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confirmed by the results of STOIC study. In spite of that, some other groups of researchers have denied such a concept (27).

There is one more procedure that presents hemodialysis, with the help of which cytokines are removed from the body during the cytokine storm. The method is called Citosorb, but unfortunately, it is expensive and it is not suitable for mass usage (28).

Antibiotic therapy

The use of antibiotics is considered necessary in ARDS or according to the antibiogram or in the treatment of already developed secondary bacterial infection.

Possible perspectives in the treatment of Covid-19

There has never been such dynamism in the scientific and professional attempts and so much research in the pharmacological world. The information comes one after another and currently at least 70 old or new drugs are being examined around the world with the possibility of administration in Covid-19 infection. At least 400 clinical studies are in progress now. In the sense of therapy, we are still in the complete dark, with little, but insufficient advances, expecting the appearance of vaccine and specific antiviral therapy.

The Prevention of Covid-19

Supplementation and possible prevention of this disease is the subject of constant interest. The preventive role of high doses of vitamin D is indisputable (10.000 IU a day during a few weeks, and after that 5.000 IU can be administered daily). Vitamin D has its receptors on the cells of inborn immunity and acquired immunity as well. It induces the creation of antimicrobial proteins, alleviates the cytokine storm and the synthesis of pro-inflammatory cytokines, it alleviates the conditions, which as comorbidities worsen Covid-19 and it has numerous other therapeutic effects (29).

Due to the reduction of pro-inflammatory influence and subepithelial local metabolic inflammation in the bowels, which is a precondition of systemic inflammation, probiotics are deemed to be rational prevention and therapy, as well (30).

Zinc (at least 15 mg a day) evidently prevents the virus replication, polyunsaturated fatty acids reduce the synthesis of pro-inflammatory substances, vitamin C (500 mg) has the antioxidant and immunomodulatory effect, alpha lipoic acid (300 to 600 mg a day) has a neuroprotective effect, improves and prevents anosmia etc.

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PCR TESTIRANJE NA SARS-CoV-2: PRAKSA, PREPORUKE I NEDOUMICE

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SAŽETAK

Pandemija Kovid-19 je pred dijagnostičke laboratorije postavila nove zahteve koji su višestruko premašili postojeće kadrovske, tehničke i materijalne kapacitete. Prema važećim preporukama, osnova laboratorijske dijagnostike Kovid-19 je *Real-Time Reverse Transcription Polymerase Chain Reaction* (rtRT-PCR) kojim se detektuje prisustvo genoma SARS-CoV-2 u biološkom materijalu uzetom od pacijenta. Postupak testiranja se uslovno može podeliti u nekoliko faza: 1) postavljanje indikacija za testiranje; 2) uzimanje kliničkih uzoraka i uvođenje u evidenciju; 3) transport materijala do dijagnostičke laboratorije; 4) prijem i razvrstavanje materijala; 5) obrada uzoraka nakon prijema – priprema za rtRT-PCR; 6) rtRT-PCR; 7) saopštavanje rezultata. Jasno je da medicinske mikrobiološke službe nisu normirane za borbu protiv pandemije ili velikih epidemija. Najefikasniji način da se prevaziđe takva situacija je da se definišu timovi koji bi se uključivali u dijagnostiku kada to epidemiološka situacija nalaže. Potrebno je težiti da oprema bude unificirana na celom prostoru Republike Srbije, jer se na taj način olakšava održavanje, nabavka rezervnih delova i potrošnih materijala, a verovatno može da se umanji i nabavna cena. Reagensi za ekstrakciju i specifični reagensi za rtRT-PCR (prajmeri i probe) treba da budu standardizovani i domaćeg porekla kako bi se umanjila zavisnost od uvoza i značajno uštedeo novac. Baza podataka laboratorijskih rezultata treba da bude unapređena i organizovana na FAIR (*findability, accessibility, interoperability and reusability*) principima kako bi se maksimalno iskoristila mogućnost izvođenja širih zaključaka.

Ključne reči: Kovid-19, rtRT-PCR, preporuke eksperta

Pandemija Kovid-19

Pandemija Kovid-19 izazvana širenjem korona virusa SARS-CoV-2 (teški akutni respiratorni sindrom koronavirus 2) sa više od dvadeset miliona zaraženih i više od osam stotina hiljada preminulih je nanela neizmerne štete gotovo svim aspektima uobičajenog života u savremenom društvu (1). Pred dijagnostičke laboratorije je postavila nove zahteve koji su višestruko premašili postojeće kadrovske, tehničke i materijalne kapacitete. Prema važećim preporukama, osnova laboratorijske dijagnostike Kovid-19 je *Real-Time Reverse Transcription Polymerase Chain Reaction* (rtRT-PCR) kojim se detektuje prisustvo genoma SARS-CoV-2 u biološkom materijalu uzetom od pacijenta (2). Postupak testiranja se uslovno može podeliti u nekoliko faza: 1) postavljanje indikacija za testiranje; 2) uzimanje materijala i uvođenje u evidenciju; 3) transport materijala do dijagnostičke laboratorije; 4) prijem i razvrstavanje materijala; 5) obrada uzoraka nakon prijema – priprema za rtRT-PCR; 6) rtRT-PCR; 7) saopštavanje rezultata. Pored ovih faza koje su direktno vezane za testiranje potrebno je u celini sagledati još neke aspekte bez kojih je nemoguće sprovesti testiranje, a to su kadrovi koji izvode testove, nabavka svih potrebnih materijala (i lične zaštitne opreme) koji se koriste u testiranju.

Cilj ovog rada je da se opiše lično ikustvo stečeno kroz sve pomenute faze, kao i da se iznesu predlozi za poboljšanje postojećih procesa i neke od nedoumica.

Postavljanje indikacija za testiranje na Kovid-19

Ova faza malo zavisi od ljudstva u laboratoriji i njihov doprinos u donošenju odluka je zanemarljiv. Testiraju se osobe koje imaju kliničke simptome i znake akutne respiratorne infekcije, njihovi kontakti, lekari i drugo

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PCR TESTING FOR SARS-CoV-2: PRACTICE, RECCOMMENDATIONS AND DILEMMAS

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SUMMARY

In the Covid-19 pandemic, diagnostic laboratories have met multiple new demands, which have gone beyond the existing personnel, technical and material capacities. According to the current recommendations, the base of laboratory diagnostics for Covid-19 is a Real-Time Reverse Transcription Polymerase Chain Reaction (rtRT-PCR) test, which detects the presence of SARS-CoV-2 genome in the biological material collected from the patient. The procedure of testing can conditionally be divided into several stages: 1) setting the indications for testing; 2) collecting the clinical samples and filling in the documentation; 3) transport of the material to the diagnostic laboratory; 4) reception and classification of the material; 5) the analysis of samples after the reception – the preparation for rtRT-PCR; 6) rtRT-PCR; 7) communicating results. It is clear that medical microbiological services have not been standardized for the fight against the pandemic or great epidemics. The most efficient way to overcome such a situation is to define teams, which would take part in the diagnostics, when the epidemiological situation demanded it. It is necessary to strive to have the unified equipment on the whole territory of The Republic of Serbia, because in that way the maintenance and procurement of spare parts and consumables are made easier, and the purchasing price could possibly be lowered. Reagents for the extraction and specific reagents for rtRT-PCR (primers and probes) should be standardized and made in Serbia in order to decrease the dependence on imports and make significant money savings. The database of laboratory results should be improved and organized according to the FAIR (findability, accessibility, interoperability, and reusability) principles in order to use the possibility of making conclusions maximally.

Keywords: Covid-19, rtRT-PCR, expert's recommendations

The Covid-19 pandemic

The Covid-19 pandemic caused by the spread of coronavirus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) with more than twenty million infected people and more than eight hundred thousand deaths, has caused immeasurable harm to almost all aspects of everyday life in the contemporary society (1). Diagnostic laboratories have met new demands, which have gone beyond the existing personnel, technical and material capacities. According to the current recommendations, the base of laboratory diagnostics of Covid-19 is *Real-Time Reverse Transcription Polymerase Chain Reaction* (rtRT-PCR), which detects the presence of SARS-CoV-2 genome in the biological material collected from the patient (2). The procedure of testing can conditionally be divided into several stages: 1) setting the indications for testing; 2) collecting the clinical samples and filling in the documentation; 3) transport of the material to the diagnostic laboratory; 4) reception and classification of the material; 5) the analysis of samples after the reception – the preparation for rtRT-PCR; 6) rtRT-PCR; 7) communicating results. In addition to these stages, which are directly connected with testing, some other aspects should necessarily be taken into consideration, because without them testing cannot be conducted, and they include the personnel, who run tests, and the procurement of all the necessary materials (including the personal protective equipment), which are used during testing.

medicinsko i nemedicinsko osoblje koje je bilo u kontaktu sa obolelima ili infektivnim materijalom, službenici organa reda i pripadnici vojske koji su bili u kontaktu sa zaraženim osobama i, u zavisnosti od epidemiološke procene, osobe koje dolaze iz prekograničnih žarišta infekcije. U poslednje vreme nameće se velika potreba za testiranjem osoba koje bi želele da putuju u zemlje čija administracija zahteva negativan rtRT-PCR rezultat za prelazak državne granice. Ostaje pitanje da li bi trebalo uvesti obavezno periodično testiranje za osobe koje rade u kontaktu sa decom kao što su zaposleni u predškolskim ustanovama, osnovnim i srednjim školama, fakultetima i službama socijalne zaštite.

Uzimanje materijala i uvođenje u evidenciju

Uzimanje materijala je jednako važno kao i svaka druga faza u procesu testiranja i u njoj ima najviše prostora za unapređenje i poboljšanje organizacije. Uzorkovanje se vrši na više stotina mesta u Srbiji i, nažalost, nije uniformno što veoma otežava postupke u laboratorijskom testiranju. Najidealnije postupak treba da se realizuje na sledeći način. Po prijemu pacijenta, na mestu uzorkovanja, trebalo bi da se unese njegov matični broj u Kovid-19 bazu, da se pripremi posuda/epruveta sa nalivenim transportnim medijumom i sa zalepljenom šestoznakovnom nalepnicom i bar-kodom (jedinstvenim za čitavu zemlju) te da se imenu pacijenta u Kovid-19 bazi pridruži šestoznakovna šifra pomoću bar-kod čitača, a zatim da se opredeli u koju laboratoriju se uzorak šalje. Ovo je veoma važno, jer greška pri manuelnom unošenju (bar-koda, a zatim imena i prezimena, čak pisma kojim je ime ubačeno, JMBG-a) može da onemogući kasniju identifikaciju uzorka i prouzrokuje velika kašnjenja ili onemogući izdavanje validnih rezultata. Pogrešan izbor laboratorije u koju se uzorak upućuje veoma otežava, a u slučaju velikog opterećenja dijagnostičke laboratorije, u potpunosti onemogućava pravovremenu identifikaciju i obradu uzorka.

Uzimanje uzoraka bi trebalo da se vrši pomoću brisa od dakrona, rejona ili viskoze na tankom plastičnom štapiću sa obaveznom tačkom lomljenja, negde na 45 mm od vrha brisa. Nepostojanje tačke prekida često može voditi teškoćama prilikom unošenja i zalomljenja brisa u epruvetu te mogućoj kontaminaciji okoline. Bris bi trebao da bude uzet iz predela nazofarinksa. Praksa je, naročito u početku, bila potpuno drugačija. Uzimani su bris ždrela i nosa pomoću dva drvena štapića na koje je bila namotana vata. Takvi brisevi nisu adekvatni jer drvo može da sadrži inhibitore sledstvenih enzimskih reakcija, pamuk nije adekvatan za uzimanje materijala za pretragu na viruse PCR metodom, a mesta sa kojih su uzimani brisevi (nos i ždrelo) nisu najpogodnija za dijagnostiku SARS-CoV-2.

Važno je pomenuti i posude sa transportnim medijumom i sam transportni medijum u koji su brisevi kasnije potapaju. Idealno bi bilo da se virusni transportni medijum (VTM) 1,5-2 ml sipa u posude nalik na *Bijou* kontejnere (zapremina 5-6 ml, visine oko 50 mm i unutrašnjeg prečnika oko 15 mm sa poklopcem koji dihtuje), jer obrada uzorka iz takvih posuda izrazito ubrzava proces dalje obrade. Korišćenje posuda od 50 ml ili epruveta od 15 ml, standardnih epruveta 12 × 75 mm i raznih drugih kreativnih i iznuđenih rešenja otežava i usporava dalje postupke obrade uzoraka. Dovoljno je reći da svako zaranjanje pipete sa nastavkom u posudu čiji je zid viši od dužine nastavka nosi opasnost od kontaminacije pipete i zahteva njenu dekontaminaciju nakon svakog uzorkovanja što jako otežava rad i usporava proces dijagnostike.

Što se tiče tečnosti u koju se brisevi potapaju najbolje bi bilo da se koristi VTM koji proizvodi Torlak, jer je kompatibilan sa svim metodama dalje obrade uzorka. U idealnom slučaju *Bijou* kontejneri bi se punili na Torlaku odgovarajućom količinom VTM obeleženi nalepnicom sa šestoznakovnom šifrom i bar-kodom i zajedno sa brisevima od pogodnih materijala, na štapićima lomljivim na 45 mm od vrha, distribuirali svim mestima za uzorkovanje.

Uzorkovanje može da se vrši u prostoriji ili na otvorenom. Uzorkovanje na otvorenom je praktično, međutim nije moguće da se sprovodi u svim vremenskim uslovima. Uzorkovanje u prostorijama može da se obavlja u zdravstvenim ustanovama, ili izvan njih, na terenu. Ostaje dilema da li bi u slučaju uzorkovanja u zdravstvenim ustanovama bilo potrebno da se uzimaju ambijentalni uzorci kao kontrola uzorkovanja.

The aim of this study was to describe the personal experience, gained during the above-mentioned stages, as well as to put forward proposals for the improvement of existing processes and some dilemmas.

Setting the indications for Covid-19 testing

This stage does not depend much on the personnel in the laboratory and their contribution to decision-making is negligible. People, who have clinical symptoms and signs of acute respiratory infection, their contacts, doctors and other medical and non-medical personnel, who have been in contact with patients or infectious material, police officers and members of the army, who have been in contact with infected people, and depending on the epidemiological estimates, people who come from the cross-border focus of infection, are tested. There has been a great need recently for testing people, who would like to travel to countries, whose administration demands a negative rtRT-PCR result to cross the border. The question remains whether periodical compulsory testing should be introduced for people who work with children, that is, people who are employed in pre-school institutions, primary schools, high schools, faculties, and social protection services.

Collecting the material and filling in the documentation

Collecting the material is as important as all the other stages in the process of testing and this stage, most space is left for the progress and improvement of the organization. Collecting the samples is performed in more than a hundred places in Serbia, and unfortunately, it is not a uniform process, and therefore, it hinders the procedure of laboratory testing. The most ideal procedure should be performed in the following way: after admission, at the place of sample collection, the patient's personal identification number should be entered into the Covid-19 base, the container/test tube should be prepared with the filled transport medium and six-digit label with the barcode (unique for the whole country) should be stuck onto it, and then six-digit code with the help of barcode scanner should be added to the patient's name in the Covid-19 database, and one should choose to which laboratory the sample will be sent. This is very important because mistakes during the manual entry (of the barcode, name, surname, even the alphabet which was used to write, personal ID number) can make it impossible to identify the sample, give valid results or cause great delays. The wrong choice of the laboratory, which the sample is directed to, can be very hindering, and due to the great burden put on diagnostic laboratories, timely identification and analysis of the sample become completely impossible.

The collection of samples should be done with the swab made of dacron, rayon, viscose on a thin plastic shaft with the compulsory breakpoint, somewhere at 45 mm from the tip of the swab. The absence of this breakpoint can lead to difficulties when placing the swab into the tube and breaking it off and therefore, to the possible contamination of the environment. The swab should be collected from the region of the nasopharynx. The practice, especially at the beginning, was completely different. A throat swab and a nose swab were collected with the help of two wooden shafts with cotton buds. Such swabs are inappropriate because wood can contain the inhibitors of ensuing enzyme reactions, cotton is not an appropriate material for collecting the material for testing viruses with the help of the PCR method, and areas (throat and nose), which the swabs were collected from, are not the most suitable areas for the diagnostics of SARS-CoV-2.

It is important to mention the containers with the transport medium and the transport medium itself, which the swabs are inserted into. It would be ideal to pour the viral transport medium (VTM) 1.5-2 ml into containers similar to *Bijou* containers (volume 5-6 ml, with a height of about 50 mm and an inner diameter of about 15 mm with the lid which has good seals) because the analysis of samples from such containers accelerates the process of further analysis. The use of containers of 50 ml or test tubes of 15 ml, standard test tubes 12 × 75 mm and other creative and imposed solutions hinder and slow down further processes of sample analysis. It would be enough to say that the insertion of pipette tips into the container, whose wall is higher than the length of the pipette tip, bears the danger of pipette's contamination and it

Transport kliničkih uzoraka do dijagnostičke laboratorije

Nakon uzimanja brisa, i potapanja u transportni medijum, uzorci se čuvaju u frižideru na 2-8°C, ne duže od 24 časa, a zatim šalju u laboratoriju. Uzorci treba da budu propisno zatvoreni, svaki u zasebnoj zip-lok kesici, i da se transportuju uspravno, po mogućustvu u stalcima, najbolje u grupama od 12 uzoraka, sa složenim pratećim listama (ukoliko postoje), a obavezno sa listom svih uzoraka koja treba da bude identična otpremnici generisanoj iz Kovid-19 baze. Tokom transporta mora da se obezbedi temperatura 2-8°C što može da se postigne pomoću ručnih frižidera za transport i leda, ili toplotnih puferskih pakovanja. Prilikom transporta, frižider sa uzorcima mora da bude učvršćen na takav način da ne dođe do prevrtanja. Od momenta preuzimanja frižidera sa uzorcima, tokom transporta i predaje uzoraka vozač je dužan da se pridržava svih mera lične zaštite. U praksi, uzorci dolaze bez stalaka, često umotani u dve rukavice što veoma usporava proces prijema i razvrstavanja uzoraka u dijagnostičkim laboratorijama, a potencijalno može da izazove kontaminaciju prostora i osoblja na prijemu.

Najbolje bi bilo da se uzorci transportuju u stalcima po 12 (ukoliko ne postoje, treba ih napraviti od klirita isečenog na CNC (engl. *Computer Numerical Control*) mašinama, nije skupo, a jako olakšava razvrstavanje i obradu), uz odštampanu otpremnicu u ručnim frižiderima sa hladnim pakovanjima za višekratnu upotrebu.

Prijem i razvrstavanje kliničkih uzoraka

Prijem i razvrstavanje kliničkih uzoraka je jedna od dve faze u kojima dolazi do najvećeg zagušenja u procesu testiranja. Postupak u različitim laboratorijama se bitno razlikuje. U pojedinim laboratorijama (npr. Torlak) svaki uzorak se unosi ručno u knjigu prijema i dodeljuje mu se broj laboratorijskog protokola (flomasterom se ispisuje na posudu sa brisom), liste se uparuju sa uzorcima i dodeljuje im se isti broj, a nakon toga se uzorak šalje na dalju obradu.

U drugim laboratorijama (npr. laboratorija Vatreno oko, Beograd) uzorci i svi prateći materijali se odmah po prispeću inaktivišu toplotom temperature 60-70°C u trajanju 45-60 minuta, a zatim se pomoću bar-kod skenera praktično potvrđuju prijemi uzoraka i upoređu sa elektronskom listom uzoraka iz otpremnica kreiranih u Kovid-19 bazi, da bi se dalje slali na ekstrakciju pri čemu je šestoznakovna šifra ujedno i broj laboratorijskog protokola.

Kasnije je služba prijema na Torlaku implementirala primenu bar-kod čitača i unosila broj protokola elektronski u Excel tabelu, ali je nastavljeno pisanje laboratorijskih brojeva protokola na posudama sa transportnim medijumom i listama.

U ovoj fazi je neophodna priprema i obeležavanje potrebanog broja epruveta za dalju obradu (može da varira 1-3), kao i kriotube koja služi za dugotrajno čuvanje transportnog medijuma u zamrzivačima na -80°C. U laboratoriji Vatreno oko, prilikom izolacije je pomoću bar-kod čitača uzorak unošen u tačnu poziciju na mikrotitar ploči za automatsku izolaciju robotom *(deep-well* ploča), a sama ploča je dobijala svoj jedinstveni bar-kod koji je štampan u triplikatu (za mikrotitar ploču za alikvote uzoraka, mikrotitar ploču sa izolatima RNK i mikrotitar ploču za rtRT-PCR).

Svakako bi bilo ispravnije da uzorak odmah dobije laboratorijski broj protokola, jer je izuzetno koristan kad se dodeljuje sukcesivno na prijemu i olakšava nalaženje, identifikaciju i ponavljanje obrade uzorka u slučaju da se za tim pojavi potreba, ali nisam siguran da su liste neophodne i koja je njihova prednost u odnosu na isključivo oslanjanje na elektronsku evidenciju. Sa druge strane, vođenje knjige protokola je, osim kad bi bilo elektronsko i automatsko, suvišno, jer preopterećuje ljudstvo na prijemu i u praksi otvara mogućnost za nastanak grešaka. Laboratorijski broj protokola je izuzetno korisno obeležje uzorka u situaciji u kojoj se ne koriste bar-kod čitači jer se dodeljuje sukcesivno na prijemu i olakšava nalaženje, identifikaciju i ponavljanje obrade uzorka u slučaju da se za tim pojavi potreba.

Inaktivacija uzoraka je takođe osetljivo pitanje. Svakako, to je deo rutinske procedure u laboratorijama Vatreno oko u koje brisevi stižu u posebno formulisanom transportnom medijumu, ekstrakcija se vrši MGI reagensima, a rtRT-PCR pomoću reagensa BGI proizvođača, a razlog verovatno leži u smanjenju opasnosti

demands decontamination after each sampling, which hinders and slows down the diagnostic process.

When we consider liquids, which the swabs are inserted into, VTM, produced by "Torlak", is recommended as the best solution because it is compatible with all the methods of further sample analysis. In the ideal case, *Bijou* containers would be filled at the Institute "Torlak" with the suitable quantity of VTM and labeled with a six-digit code and barcode, and together with swabs made of appropriate materials, on shafts with the breakpoint at 45 mm from the tip, would be distributed to all places, where samples are collected.

Sampling can be performed inside or in the open space. Sampling in the open space is practical; however, it is not possible in all weather conditions. Sampling can be performed in the rooms within the health care institutions or outside of them, on the terrain. The dilemma remains whether in case of sampling in health care institutions it would be necessary to collect ambiance samples as the means of sampling control.

The transport of clinical samples to diagnostic laboratories

After swabs are collected and inserted into the transport medium, the samples are kept in the fridge at 2-8°C for no longer than 24 hours, and then they are sent to the laboratory. Samples should be properly closed, each in a separate zip lock bag, and transported in the upper position, possibly in stands, best in groups of 12 samples, with complex lists (if they exist), and obligatorily with the list of all samples which should be identical to the delivery note generated from the Covid-19 database. During transport, the temperature of 2-8°C should be provided, which could be achieved by hand fridges for the transport of ice or heat-resistant buffer packaging. During transport, the fridge with samples should be tightened in order not to be turned over. The driver has to respect all the measures of personal protection when he takes the fridge with samples, during transport, and when he hands over the fridge. In practice, samples come without stands, they are often wrapped in two gloves, and this slows down the process of reception and classification in diagnostic laboratories, and potentially this can cause the contamination of space and personnel during the reception.

It would be best to transport 12 samples in stands (if there are no stands, they should be made of clirite cut on CNC (Computer Numerical Control) machines; this is not expensive and it makes the classification and analysis easier), with the printed delivery note in hand fridges with cold packages for the repeated use.

The reception and classification of samples

The reception and classification of clinical samples is one of the two phases, when the process of testing becomes clogged. The procedure significantly differs in different laboratories. In some laboratories (e.g. "Torlak), each sample is entered manually into the book of reception and the number of laboratory protocol is assigned to it (it is written with a marker on the container with the swab), the lists are matched with the samples and the same number is assigned to them, after which the specimen is sent to further processing.

In other laboratories (e.g. laboratory "Fiery Eye", Belgrade), samples and all the materials are immediately upon reception inactivated at temperature 60-70°C lasting 45-60 minutes, and then with the help of barcode scanners the reception of samples is confirmed and they are compared with the electronic mailing lists of samples from delivery notes created in the Covid-19 database, so that they could be sent to extraction, while the six-digit code is at the same time the number of laboratory protocol.

Later, the service of reception at the Institute "Torlak" implemented the barcode scanner and entered the number of protocol electronically into the *Excel* table, but they continued to write the numbers of laboratory protocols on containers with transport medium and on the lists.

During this stage, it is necessary to prepare and mark the number of test tubes needed for further processing (it can vary from 1-3), as well as cryotube, which is used for the long-term maintenance of transport medium in freezers at -80°C. In the laboratory "Fiery Eye", during the isolation with the help of a barcode scanner, the

od infekcije u ambijentu sa više hiljada analiziranih uzoraka dnevno. Ipak, skromni paralelni eksperiment sa relativno malim brojem uzoraka (nije bilo dovoljno vremena da se testira veliki broj uzoraka paralelno) iz VTM uz lizu sa *SanSure release* reagensom i rtRT-PCR, korišćenim prema uputstvu iz *SanSure* kita, pokazao je da se nakon inaktivacije u vodenom kupatilu u trajanju od 45 min na 56°C drastično smanjuje broj pozitivnih uzoraka. To znači da je neophodno paralelni eksperiment ponoviti na većem broju poznatih uzoraka sa svakom novom vrstom transportnog medijuma, svakom novom vrstom obrade uzorka, kao i sa svakim novim kitom za detekciju (validacija metode).

Veoma je važno da se obezbedi ljudstvo za obeležavanje epruveta i njihovo slaganje u stalke kako bi se olakšala dalja obrada uzoraka. Obeležavanje epruveta mora da bude čitko, tankim flomasterima koji se teško brišu sa polipropilena, može da se vrši u prostorima koji nisu namenjeni za rad sa infektivnim materijalom i da se obeležene epruvete sukcesivno dostavljaju u laboratoriju za obradu materijala. Štampanje jedinstvenog bar-koda u više primeraka i korišćenje bar-kod čitača (za svaki sukcesivni korak obrade) u mnogome bi olakšalo datu proceduru. Prazni stalci se nakon dezinfekcije i sušenja vraćaju nazad u prostoriju gde se obeležavaju epruvete. U laboratorijama Vatreno oko se u obradi uzorka ne koriste epruvete nego *deep-well* ploče sa 96 mesta koje se jedinstveno obeležavaju što bitno olakšava postupak i gotovo u potpunosti eliminiše potrebu za obeležavanjem epruveta, osim krio-tuba.

Neophodno je preporučiti korišćenje *deep-well* ploča, isto bi bilo potrebno eksperimentalno odrediti vreme centrifugiranja kada se obrada uzoraka vrši pomoću reagenasa za lizu, jer se za ove ploče koriste *swinging bucket* rotori koji ne mogu da pruže dovoljno veliku brzinu centrifugiranja za razliku od *fixed-angle* rotora za epruvete. Razlog je lakši rad, smanjenje grešaka u obeležavanju i pozicioniranju uzorka prilikom postavljanja u rotor i vraćanja u stalak, kao i ubrzavanje rada tokom obrade korišćenjem multikanalnih pipeta. Takođe, neophodna je intenzivna obuka kadra na prijemu za rad u *Excel*-u jer sam lično imao prilike da se uverim da znanje za korišćenje tog programa, veoma blago rečeno, nezadovoljavajuće, iako, makar među mlađim laborantima to ne bi smeo da bude slučaj s obzirom da se korišćenje *Excel*-a, na mnogo višem nivou, uči u drugom razredu srednje škole.

Još jedan problem predstavljaju pritisci koji dolaze spolja da se pojedini uzorci preko reda obrade. Svi smo svesni da je nemoguće da se takva praksa u potpunosti iskoreni, ali osoblje koje razvrstava uzorke nikako ne treba da bude izloženo toj pojavi. Ukoliko postoji potreba da se iz nekog razloga neki uzorci obrade preko reda, onda je neophodno da šef ekipe identifikuje te uzorke i donese ih iz kofera na razvrstavanje i dodeljivanje broja laboratorijskog protokola. U protivnom, može da dođe do prevelikog remećenja i usporavanja procesa dijagnostike i nastanka grešaka kakve su npr. preskakanje ili dupliranje brojeva.

Obrada uzoraka i priprema za rtRT-PCR

Obrada uzoraka i priprema za rtRT-PCR može da se odvija u dva pravca. To su 1) liza sedimenta transportnog medijuma i 2) ekstrakcija nukleinskih kiselina (NK) iz transportnog medijuma. Prednost lize je brzina izvođenja (moguće i niža cena), a prednost ekstrakcije NK je čistoća uzorka koji se amplifikuje. Nezavisno od toga da li se radi liza ili ekstrakcija NK, potrebno je da se automatskom pipetom prenese zadata količina transportnog medijuma u epruvetu ili *deep-well* ploču i u krio-tubu u kojoj se čuvaju smrznuti uzorci. Veoma je važno da se posude sa brisom pre uzimanja uzorka za dalju obradu snažno promućkaju (poželjno na vorteksu) da bi se uzorak homogenizovao. Ukoliko se uzorak nalazi u uskim posudama (<25 mm unutrašnjeg prečnika) sa zidom višim od 60 mm neizostavno se javljaju komplikacije. Kontaminacija automatske pipete je izvesna, što veoma usporava rad, jer posle svakog uzorka pipeta mora da se dekontaminira i povećava se mogućnost unakrsne kontaminacije uzoraka. To može da se prevaziđe tako što se uzorak prvo prenese Pasterovom/jednokratnom transfer pipetom u krio-tubu pa se zatim odatle pipetira potrebna količina uzorka za dalju obradu. Ono što može da predstavlja problem to je da se Pasterove pipete pojedinačno pakuju što zahteva otvaranje svake pojedinačno, dovodi do velikog gubitka vremena i stvara velike količine

specimen was placed into the correct position on the microtitre plate for the automated isolation with the help of a robot (deep-well plate), and the plate itself was given a unique barcode, which was printed as a triplicate (for the microtitre plate for the aliquots of samples, microtitre plate with RNK isolates, and microtitre plate for rtRT-PCR).

Certainly, it would be more correct to assign the laboratory number of protocol to the specimen, because it is useful when it is assigned successively upon reception and it facilitates finding, identifying, and repeating the processing of specimen if the need appears. However, I am not sure whether the lists are necessary and what their advantage in comparison to electronic documentation is. On the other hand, keeping the book of protocol, except if it was electronic and automatic, is needless because it overburdens the personnel at reception, and in practice, it opens up the possibility of making mistakes. The laboratory number of the protocol is an extremely useful mark of the specimen when barcode scanners are not used because it is assigned successively upon reception and it facilitates finding, identifying, and repeating the processing of sample if it is necessary.

The inactivation of samples is also a touchy question. Certainly, it is part of the routine procedure in the laboratory "Fiery Eye", to which swabs are delivered in the specially formulated transport medium, the extraction is done with MGI reagents, while rtRT-PCR is done with the help of reagents of BGI producer, and the reason probably lies in the decrease of risk of infection in the ambiance, in which a few thousand samples are analyzed on a daily basis. However, a modest parallel experiment with a relatively small number of samples (there was not enough time to test a great number of samples in parallel) from the VTM with the *SanSure* release reagent and rtRT-PCR, used according to the instructions from the *SanSure* kit, showed that after the inactivation in the water bathroom lasting 45 minutes at 56°C drastically reduced the number of positive samples. It means that the parallel experiment should be repeated on a larger number of known samples with new types of transport medium, new types of sample processing, as well as with new detection kits (method validation).

It is important to provide personnel, who would mark the test tubes, and place them into stands, so that further processing of samples would be made easier. Test tubes should be marked legibly, with thin markers, which are hard to be erased from polypropylene. It can be done in rooms, which are not intended for work with infectious materials and marked test tubes can successively be delivered to the laboratory for sample processing. Printing the unique barcode in more copies and using the barcode scanner (for each successive step of processing) would make the given procedure easier. After disinfection and drying, empty stands are returned to the room, where test tubes are marked. In the laboratory "Fiery Eye", test tubes are not used for sample processing, but deep-well plates with 96 spaces, which are uniquely marked, which facilitates the procedure and almost completely eliminates the need to mark the test tubes, except cryotubes.

It is necessary to recommend the use of deep-well plates, although the time of centrifugation should be determined experimentally when the sample processing is done with the help of reagents for lysis, because swinging bucket rotors are used for these plates and they cannot offer sufficiently great speed of centrifugation in comparison to fixed-angle rotors for test tubes. The reason is the easier work, and the reduction of mistakes regarding marking and positioning of samples during their placement into the rotor and back to the stand, as well as faster processing with the help of multichannel pipettes. Also, it is necessary to train the personnel at reception to work in *Excel*, because I had the chance to see that knowledge about that program is unsatisfactory, although at least among younger laboratory technicians this should not be the case because the use of *Excel* is studied in the second year of high school, but at a lot higher level.

One more problem is pressure from outside to analyze some samples out of turn. We are all aware of the fact that such practice cannot be eradicated completely, but personnel, who classify the samples, should not be exposed to such occurrence. If there is a need to analyze some samples out of turn, then it is necessary that the head of that team identifies those samples and brings them from the suitcase so that they could

otpada koji se tretira kao infektivan jer se otvara u laminarnoj komori. Druga mogućnost je da se uzorak transportnog medijuma dekantuje u krio-tubu što sa sobom nosi opasnost od prosipanja uzoraka i kontaminacije zaštitne opreme i radne površine i opreme.

Brzina obrade je varirala u zavisnosti od primenjene tehnologije obrade. Moja iskustva sa *SanSure release* reagensom su takva da mogu da tvrdim da dobar laboratorijski tehničar može da obradi oko tri stotine uzoraka za radno vreme od šest sati, pod uslovom da dobija obeleženu epruvetu u koju sipa uzorak za centrifugiranje i obeleženu krio-tubu u koju sipa uzorak za čuvanje. Dalji uslov je da dobija uzorke brisa u posudama koje su nalik *Bijou* bočicama. Međutim, nisu svi uzorci bili pogodni za rad sa *SanSure release* reagensom. Određeni uzorci koji su bili prebačeni iz laboratorije Vatreno oko u Beogradu u epruvetama kineske proizvodnje nisu mogli da se obrađuju na ovaj način. Ostalo je neutvrđeno da li je to zbog toga što je transportni medijum nepogodan za lizu (sadrži SDS) pomoću *SanSure release* reagensa i rtRT-PCR sa *SanSure master mix*-om, ili je to zbog termičke inaktivacije uzoraka ili iz nekog drugog razloga. U nedostatku *SanSure* reagenasa liza uzoraka iz VTM je uspešno izvođena pomoću *Arcis* reagensa za lizu i *GeneFinder* i *DAAN* kitova za rtRT-PCR.

Ekstrakcija NK u dijagnostici Kovid-19 je vršena na više načina. Ja sam imao prilike da vršim izolaciju pomoću kolona na *Qiacube* automatskom ekstraktoru, različitim *Qiagen* kitovima sa kolonicama koje se centrifugiraju, *Vector* reagensima za manuelnu ekstrakciju, MGI reagensima (ručna ekstrakcija) i *MagMax* reagensima (ručna ekstrakcija). Zajednički imenitelj svim tim metodama je mali broj uzoraka koji mogu da se obrade (varira od 60 do 96 u toku šest časova). Svakako prednost dajem magnetnoj ekstrakciji u slučaju da je dnevno potrebno da se obradi veliki broj uzoraka u odnosu na kolone, automatsku *Qiacube* ekstrakciju i precipitaciju alkoholom (*Vector*). Zajedničko ovim metodama je i to što zahtevaju značajno višu obučenost kadra i mnogo više manuelnog rada (osim ekstrakcije na *Qiacube* aparatu). Cena ekstrakcije je značajno viša kada se koriste kolonice nego magnetne kuglice ili precipitacija alkoholom. Takođe, cena potrošnog materijala (tj. broj nastavaka sa filterom) je viša kada se radi ekstrakcija NK nego kada se radi liza.

U laboratoriji Vatreno oko izolacija NK je bazirana na automatskoj izolaciji magnetnim kuglicama MGI kitovima u MGISP-960 automatizovanom robot sistemu. Ovaj pristup omogućavao je da u procesu od 90 minuta (uključujući i dekontaminaciju aparata) budu izolovane i spremne za rtRT-PCR dve mikrotitar ploče sa uzorcima (188 uzoraka), što umnogome ubrzava proces detekcije virusa i saopštavanje rezultata.

Bilo bi dobro, a i izvodljivo je, proizvesti reagense i odgovarajuće magnetne stalke, kao i potrebnu opremu, u Srbiji, što bi umanjilo potrebu za uvoznim komponentama u ovoj fazi testiranja. Moguće bi bilo napraviti i reagense za alkoholnu precipitaciju NK u Srbiji, ali sa takvim reagensima ne bi mogla da se postigne zadovoljavajuća brzina ekstrakcije koja odgovara potrebama tokom epidemije.

Najbolje bi bilo da se koristi magnetna ekstrakcija (ručna ili automatska po mogućstvu) i da se proizvedu reagensi i prateća oprema za ručnu izolaciju u Srbiji. Adekvatno dizajnirani magnetni nosači za *deep-well* ploče bi omogućili da jedan tehničar bez preteranog napora izoluje oko četiri stotine i osamdeset uzoraka u toku radnog vremena od šest sati. Dodatna korist je što bi izolovane NK bile u ploči formata 8 × 12 bunarčića što bi olakšalo transfer uzoraka u ploču za rtRT-PCR.

Po pravilu, uzorke bi trebalo čuvati na temperaturi od -80°C. Kapaciteti takvih zamrzivača su više nego ograničeni i apsolutno nedovoljni za čuvanje tolikog broja uzoraka. Na Torlaku su oni čuvani do popunjavanja kapaciteta na -80°C, a kasnije u hladnjači na -20°C. Nedostajale su jasne procedure šta se radi sa takvim uzorcima, i takve procedure treba svakako doneti.

Izvođenja rtRT-PCR testa za dijagnozu Kovid-19

Metodologija rtRT-PCR po pravilu podrazumeva dva koraka. Prvi korak je reverzna transkripcija, a drugi korak je PCR sa detekcijom nastalog PCR produkta u realnom vremenu. Kombinovanjem enzimskog miksa koji u sebi sadrži enzime reverznu transkriptazu i DNK

be classified and that the numbers of laboratory protocol could be assigned. On the contrary, the process of diagnostics could be disturbed and slowed down and mistakes could be made, such as skipping the numbers or duplicating them.

The processing of samples and preparation for rtRT-PCR

The processing of samples and preparation for rtRT-PCR can unfold in two directions: 1) the lysis of sediment of transport medium; and 2) the extraction of nucleic acid (NA) from the transport medium. The advantage of lysis is its speed (and possibly lower price), and the advantage of NA extraction is the purity of the amplified sample. Regardless of the fact whether lysis or NA extraction is performed, the given quantity of transport medium should necessarily be transported with the automatic pipette into the test tube or deep-well plate or cryotube, in which frozen samples are kept. It is very important to shake the containers with the swab before collecting the sample for further processing (desirably on vortex) in order to homogenize the sample. If the sample is in narrow containers (<25 mm of inner diameter) with the wall higher than 60 mm complications will appear for sure. The contamination of the automatic pipette is certain, which slows down the work because the pipette has to be decontaminated after each sample, and the possibility of cross-contamination of samples increases. It can be solved by transferring the sample first with the Pasteur/single-use transfer pipette into the cryotube, and from there the necessary quantity of sample is taken with the pipette for further processing. What can be problematic is that Pasteur pipettes are packed separately, which demands the opening of each pipette, and therefore time is wasted and great amounts of waste are created and this waste is treated as infectious because it is opened in a laminated chamber. The other possibility is to decant the sample of transport medium into the cryotube, which bears the risk of spilling the sample and contamination of protective equipment, surfaces, and equipment.

The speed of processing has varied depending on the applied technology. My experience with *SanSure* release reagent is such that I can claim that a good laboratory technician can process about three hundred samples for six hours, under condition that he gets a marked test tube, which he fills with the sample for centrifugation and a marked cryotube, which is filled with the sample for keeping. The next condition is that he gets the swab samples in containers similar to Bijou bottles. However, there were some samples which were not suitable for working with SanSure release reagent. Some samples, which were transferred from the laboratory "Fiery Eye" in Belgrade in test tubes made in China, could not be processed in this way. It has remained unclear whether this happened because the transport medium was not suitable for lysis (it contains SDS) with the help of *SanSure* release reagents and rtRT-PCR with *SanSure master mix*, or it happened due to the thermal inactivation of samples or some other reason. Due to the lack of SanSure reagents, the lysis of samples from VTM was successfully done with the help of Arcis reagents for lysis and GeneFinder and DAAN kits for rtRT-PCR.

The NA extraction in the Covid-19 diagnostics was performed in several ways. I had the chance to isolate with the help of columns on *Qiacube* automatic extractor, different *Qiagen* kits with columns that are centrifugated, Vector reagents for manual extraction, MGI reagents (manual extraction), and *MagMax* reagents (manual extraction). The common denominator of all these methods is the small number of samples that can be processed (it varies from 60 to 96 for six hours). I certainly give priority to the magnetic extraction when a large number of samples have to be processed in comparison to columns, automatic *Qiacube* extraction, and ethanol precipitation (*Vector*). What these methods have in common, is the fact that they demand significantly more trained personnel and a lot more manual work (except the extraction on *Qiacube* machine). The price of extraction is significantly higher when columns are used in comparison to magnetic beads or ethanol precipitation. Also, the price of consumables (that is, the number of tips with filters) is higher when NA extraction is performed in comparison to lysis.

In the laboratory "Fiery Eye", the isolation of NA is based on the automatic isolation with magnetic beads MGI kits in the MGISP-960

zavisnu DNK polimerazu omogućeno je da se te dve reakcije odigraju sukcesivno u istoj reakcionoj smeši. Korišćenje takvih reakcionih smeša značajno smanjuju manuelni rad, potrošnju nastavaka za automatske pipete sa filterima i ubrzavaju proces testiranja. Analiza izvedena rtRT-PCR metodom po pravilu traje između 90 i 120 minuta od momenta kada se završi pipetiranje. To znači da u šestočasovnoj smeni jedan operater može na jednoj PCR mašini da finalizuje tri ciklusa sa maksimalno 94 uzoraka po ciklusu odnosno 282 uzoraka. Lako je da se izračuna da na jednoj PCR mašini može da se dnevno izda čak nešto iznad 1.100 rezultata.

Reagensi za rtRT-PCR na SARS-CoV-2

Više od dvadeset različitih testova je bilo dostupno u Srbiji za testiranje uzoraka na SARS-CoV-2 PCR metodologijom. Svi reagensi sa kojima se radilo u Srbiji su omogućavali izvođenje reverzne transkripcije i *Real-Time* PCR-a u istoj reakcionoj smeši, osim reagenasa nabavljenog od firme *Vector*, sa kojima se reakcija odigravala u dva nezavisna koraka. Primenjena metodologija je u velikoj meri smanjivala mogućnost kontaminacije opreme i prostora, jer su se nakon završetka reakcije reakcione posude bacale neotvorene. Po pravilu, svi reagensi su u sebi sadržali oligonukleotide koji su amplifikovali jednu ljudsku sekvencu (što je predstavljalo internu pozitivnu kontrolu reakcije, IPC) i oligonukleotide koji su amplifikovali 1-3 virusne sekvence. Izuzetak je test nabavljen od firme *Vector* koji je amplifikovao samo virusnu sekvencu. Zbog inherentne nepouzdanosti takvog pristupa taj test se nije radio, osim kao probni test. Najveći broj testova je odrađen sa testovima kompanija *BGI* (IPC + jedan virusni gen), *SanSure* (IPC + dva virusna gena) i *GeneFinder* (IPC + tri virusna gena). Iako potreba da se na nivou države radi sa jednim testom možda deluje kao ograničavanje slobode tržišta, jasno je da bi korišćenje jednog testa na celoj teritoriji države imalo niz prednosti. Na prvom mestu, neizmerno bi se olakšala i ujednačila interpretacija rezultata na nacionalnom nivou, verovatno bi mogla da se postigne niža cena prilikom nabavke reagenasa uzimajući u obzir veće količine koje bi se naručile, bitno bi se povećala mogućnost poređenja efikasnosti laboratorija i olakšala fluktuacija kadra između različitih laboratorija.

Vredi napomenuti da ni za jedan od korišćenih testova nisu bile poznate sekvence i koncentracije oligonukleotida koje su ulazile u njihov sastav. Takva praksa proizvođača nije nova, ali ostaje pitanje da li Srbija kao država treba da pristane na takvu praksu. Pogotovo zbog toga što sinteza oligonukleotida nije naročito napredna tehnologija (ja sam devedesetih godina prošlog veka naručivao i radio PCR sa oligonukleotidima koji su sintetisani u tadašnjem Centru za genetski inženjering), niti kapaciteti za sintezu oligonukleotida za potrebe pandemijskog testiranja zahtevaju nabavku jako skupe opreme ili angažuju veliki broj ljudi. Dizajniranjem, sintezom i obeležavanjem oligonukleotida u Srbiji dobili bi mogućnost da proizvodimo sopstvene specifične testove, kako za Kovid-19, tako i za druge infektivne i mnoge neinfektivne bolesti.

Finalizacija reakcione smeše i plastika za rtRT-PCR na SARS-CoV-2

I u ovoj fazi testiranja su postojale velike razlike među laboratorijama. U Nacionalnoj referentnoj laboratoriji je uzorak (VTM) pipetiran direktno u PCR ploču u kojoj se nalazio *SanSure release* reagens, a zatim *SanSure master mix*. U laboratorijama Vatreno oko je uziman alikvot ekstrahovanih NK iz ploče i pipetiran automatizovanim sistemom direktno u PCR ploču sa prethodno pipetiranim master miksom. Na Torlaku je pipetiranje master miksa u ploču vršeno automatskom stanicom za pipetiranje *EpMotion 5070*, a uzorci su se iz pojedinačnih epruveta dodavali u master miks. Ostaje pitanje kako se radilo u drugim laboratorijama. U laboratorijama u kojima je obrađeno najviše uzoraka (Torlak, Vatreno oko Beograd i Nacionalna referentna laboratorija) rtRT-PCR reakcija se po pravilu izvodila u pločama. U pojedinim laboratorijama se rtRT-PCR izvodio u stripovima. Korišćenje ploča donosi nesumnjive prednosti, jer otvaranje i zatvaranje stripova povećava mogućnost kontaminacije i treba ga izbegavati. Isplativije je da se iskoristi samo četvrtina ploče nego da se rizikuje kontaminacija jednog uzorka. Utisak koji sam stekao je da se u radu nedovoljno koriste multikanalne pipete, delom zbog objektivnih okolnosti (neadekvatna

automated robotic system. This approach made it possible for 90 minutes (including the apparatus decontamination) to isolate and prepare two microtitre plates with samples (188 samples) for rtRT-PCR, which makes the process of detecting the virus and communicating results faster.

It would be good, and it is feasible, to make reagents and appropriate magnetic stands, as well as all the necessary equipment in Serbia, which would reduce the need for imported components in this stage of testing. It would be possible to make reagents for the ethanol precipitation of NA in Serbia, but with such reagents the satisfactory speed of extraction, which suits the needs during the epidemic, could not be achieved.

It would be best to use the magnetic extraction (manual or possibly automatic) and to produce the reagents and equipment for manual isolation in Serbia. Appropriately designed magnetic carriers for deep-well plates would enable a technician to isolate without much effort about four hundred and eighty samples during his shift of six hours. An additional benefit is that the isolated NA would be in the plate format 8 ×12 wells, which would make the transfer easier into the plate for rtRt-PCR.

As a rule, samples should be stored at temperature -80°C. The capacities of such freezers are more than limited and absolutely insufficient for storing such a large number of samples. At the Institute "Torlak" they were stored until capacities were filled at temperature -80°C, and then in the cold storage room at -20°C. Clear procedures on what to do with such samples were missing, and such procedures should certainly be regulated.

Running the rtRT-PCR test for the diagnosis of Covid-19

The methodology of rtRT-PCR, as a rule, includes two steps. The first step is the reverse transcription, and the second step is PCR with the detection of a PCR product in real-time. By combining the enzyme mix, which contains the enzymes reverse transcriptase and RNA dependent RNA polymerase, these two reactions could happen successively in the same reaction mixture. The use of such reactive mixtures significantly reduces the manual work, consumption of tips for automatic pipettes with filters, and makes the testing process faster. The analysis performed with rtRT-PCR method lasts between 90 and 120 minutes from the moment when the pipetting is finished. This means that during the shift of six hours one operator can finish three cycles of maximally 94 samples for one cycle, that is, 282 samples on one PCR machine. It can easily be calculated that more than 1.100 results can be issued on one PCR machine on a daily basis.

SARS-CoV-2 reagents for rtRT-PCR

More than twenty different tests have been available in Serbia for diagnostic testing for SARS-CoV-2 with the PCR method. All reagents, which have been used in Serbia, enabled running the reverse transcription and Real-Time PCR in the same reaction mixture, except in the case of reagents purchased from Vector, when the reaction was performed in two separate steps. The applied methodology reduced, to the great extent, the possibility of contamination of equipment and space because the closed containers were thrown away after the reaction. As a rule, all reagents contained oligonucleotides, which amplified one human sequence (which presented the internal positive reaction control, IPC) and oligonucleotides, which amplified 1-3 virus sequences. One exception is the test of *Vector* company, which amplified only the virus sequence. Due to the inherent unreliability of such an approach, the test was conducted only as an experimental test. The largest number of tests was conducted with *BGI* tests (IPC + one viral gene), *SanSure* tests (IPC + two viral genes), and *Gene Finder* (IPC + three viral genes). Although the need to use only one test in the whole country seems like a limitation of the freedom of the market, it is clear that the use of one test on the territory of the whole country would have its advantages. Firstly, it would standardize and facilitate the interpretation of results at the national level, and possibly lower the price because larger quantities would be ordered. The possibility of comparing the efficiency of laboratories would be increased and the fluctuation of personnel between different laboratories would be made easier.

geometrija stalaka ili nedostatak multikanalnih pipeta koje pokrivaju opseg 5-50 µl), a delom zbog subjektivnih razloga kao što su neiskustvo i nesigurnost u radu sa multikanalnim pipetama. To je velika šteta, jer korišćenje multikanalnih pipeta bitno ubrzava rad i umanjuje mogućnost greške.

PCR mašine

PCR mašine koje sam imao prilike da vidim u laboratorijama koje sam obišao su po pravilu imale dovoljan broj detektora za pokrivanje svih fluorohroma (ili njihovih spektralnih analoga) kojima su bili obeležene specifične probe. Sve PCR mašine imale su standardne blokove formata 8 × 12. Prostim računajem kapaciteta PCR mašine i dnevnih potreba testiranja u Srbiji lako se izračuna da petnaestak standardnih PCR mašina koje bi bile namenjene samo testiranju na SARS-CoV-2 uz pravilno korišćenje mogu da podmire potrebe cele Srbije, jer ni u jednom trenutku broj urađenih PCR testova nije prelazio deset hiljada. Tokom proleća 2020. godine je nabavljen određeni broj PCR mašina i tražen značajan broj novih PCR mašina i sve su bile sa standardnim blokom 8 × 12 pozicija. Svakako vredelo bi razmisliti o nabavci mašina sa izmenjivim blokom (npr. *ABI QuantStudio 6*) pri čemu bi se nabavio i termoblok formata 16 × 24 mesta, jer se tako povećava broj analiziranih uzoraka u jednom ciklusu četiri puta (384 naspram 96). Pri tome se i zapremine korišćenih reagenasa umanjuju što automatski umanjuje cenu testiranja. Smanjenje zapremine reagenasa ne bi umanjilo senzitivnost testiranja nakon optimizacija procesa obrade uzoraka i reakcionih smeša.

Ono što treba imati u vidu je i mali eksperiment koji je izvršen tokom prethodnih meseci rada na dijagnostici SARS-CoV-2 virusa, koji je, koristeći iste uzorke, odnosno iste izolate paralelno, pokazao da RT-PCR mašine različitih proizvođača imaju i različitu senzitivnost koja se ogledala u razlici čak i do 5 podeoka vrednosti Ct (engl. *Cycle threshold value*). Ovakva činjenica ima za posledicu da u zonama male koncentracije virusa detekcija targeta jednom RT-PCR mašinom može dati dijametralno suprotan rezultat od detekcije drugom, te da bi trebalo izvršiti dodatne analize i dati preporuke i u tom pogledu.

Programiranje PCR mašina za testiranje na SARS-CoV-2 metodom rtRT-PCR

Svi reagensi za rtRT-PCR testiranje na SARS-CoV-2 su imali jasno napisana uputstva za programiranje temperaturnih profila reakcije, u smislu definisanih temperatura i broja ciklusa koji su ponavljani. Međutim, ni u jednom uputstvu nisu definisane brzine dostizanja temperatura (ramp rate) što predstavlja važan PCR parametar i može da ima uticaj na ishod testiranja i reproducibilnost rezultata. Manuelno unošenje oznaka uzoraka u softver koji kontroliše mašinu je spor proces koji u sebi nosi veliku mogućnost greške. Iz tog razloga je najbolje koristiti jednostavne *Excel* templejte koji sa jedne strane olakšavaju unos, a sa druge strane olakšavaju saopštavanje rezultata. Odgovarajući *Excel* templejti su primenjivani u laboratorijama Torlak i Vatreno oko Beograd. Kreiranje tabele, odnosno liste za učitavanje brojeva uzoraka, je na Torlaku rešeno tako što se koristila *autofill* komanda u *Excel*-u (kada je god to bilo moguće), dok je u Vatrenom oku Beograd jednostavno kopiran plan ploče za ekstrakciju RNK prethodno unet u *Excel* pomoću bar-kod čitača. Moj utisak je da su se u ostalim laboratorijama koje sam obišao oznake uzoraka u softver unosile ručno tokom programiranja mašine.

Interpretacija rezultata rtRT-PCR testova na SARS-CoV-2

Interpretacija dobijenih rezultata je značajno varirala što je logična posledica korišćenja različitih testova. Najlakša interpretacija rezultata je bila kada su korišćeni testovi sa samo jednom ciljnom virusnom sekvencom (npr. *BGI*). I preporuke za tumačenje rezultata su se menjale tako da je u najnovijim protokolima preporučeno da se rezultat izdaje kao pozitivan i kada je samo jedan virusni gen bio pozitivan (najčešće N gen). Diskusije i razmena iskustava između rukovodilaca u različitim laboratorijama bi bile izuzetno korisne i doprinele bi podizanju kvaliteta interpretacije rezultata. Na žalost, nije ustanovljena praksa da se rukovodioci laboratorija periodično sastaju i diskutuju o interpretaciji rezultata, prvenstveno zbog izuzetnog napora koji je ulagan da se dijagnostika održi u potrebnom obimu, razuđenosti laboratorija i smenskog rada.

We should mention that sequences and concentration of oligonucleotides were not known for these tests. Such a manufacturer's practice is not new, but the question remains whether Serbia should accept such practice. Especially because the synthesis of oligonucleotides is not particularly advance technology (in the 1990s I ordered and conducted PCR with oligonucleotides, whose synthesis was performed in the Center for Genetic Engineering), and because capacities for the synthesis of oligonucleotides for the testing during pandemic demand very expensive equipment and engage a lot of people. If oligonucleotides were designed, marked, and synthesized in Serbia, we could make our own specific tests for Covid-19, as well as for infectious and many non-infectious diseases.

The finalization of reaction mixture and plastic for SARS-CoV-2 rtRT-PCR

In this stage of testing, there were great differences between laboratories. In the National Reference Laboratory, the sample (VTM) was placed with a pipette directly into the PCR plate, in which *SanSure* release reagent was, and then *SanSure* master mix. In the laboratory "Fiery Eye" the aliquot of extracted NA was taken from the plate and placed with the automated pipette directly into the PCR plate with a master mix, while the pipetting of master mix was previously conducted. At the Institute "Torlak", the pipetting of the master mix into the plate was conducted with the automated station for pipetting EpMotion 5070, while samples were added into the master mix from separate test tubes. The question remains how the other laboratories worked. In laboratories, in which the largest number of samples were analyzed (Torlak, Fiery Eye in Belgrade, The National Reference Laboratory), rtRT-PCR reaction was conducted in plates. In some laboratories, rtRT-PCR was conducted in strips. The use of plates has advantages, because the opening and closing of strips increase the risk of contamination and it should be avoided. It is worthwhile to use only one-fourth of the plate than to risk the contamination of the whole sample. My impression is that multichannel pipettes are not used sufficiently, partly due to objective circumstances (inadequate geometry of the stand or lack of multichannel pipettes which cover the range from 5-50 µl), and partly due to subjective reasons, such as inexperience and insecurity while working with multichannel pipettes. It is a great pity because the use of multichannel pipettes significantly makes the work faster and diminishes the possibility of mistakes.

PCR machines

PCR machines, which I had a chance to see in laboratories, had a sufficient number of detectors for covering all fluorochromes (or their spectral analogs) with which specific probes were marked. All PCR machines had standard block format 8 x 12. By simple calculation of the capacity of PCR machine and daily needs for testing in Serbia, it can easily be calculated that about fifteen standard PCR machines, which would be intended only for SARS-CoV-2 testing, would be sufficient for the needs of the whole Serbia, because the number of conducted tests never exceeded 10 thousand. In the spring of 2020, a number of PCR machines were purchased and a significant number of new PCR machines were asked for and they were all with the standard block 8 × 12 positions. It would be worthwhile to consider the purchasing of machines with the changeable block (e.g. ABI QuantStudio 6), as well as the thermal block in the format 16 × 24 places because the number of analyzed samples in the one cycle would be increased fourfold (384 in comparison to 96). Also, the volumes of reagents are reduced, which automatically lowers the price of testing. The reduction of the reagents' volume would not diminish the sensitivity of testing after the optimization of the processing of samples and reactive mixtures.

What one should have in mind is a little experiment, which was conducted during the previous months while working on the diagnostics of the SARS-CoV-2. The same samples, that is, the same isolates were used in parallel and it was shown that RT-PCR machines of different manufacturers have different sensitivity, which was reflected in the difference of even 5 cycles threshold value. This results in the fact that in the zones of a small concentration of virus, the detection of a target with one PCR machine can give a completely different result

Saopštavanje rezultata rtRT-PCR testova na SARS-CoV-2

Saopštavanje rezultata je takođe nosilo sa sobom dozu izazova i evoluiralo je u periodu između marta i avgusta. Na samom početku epidemije na Torlaku su rezultati ručno upisivani u protokol laboratorije, a zatim su izdavani odštampani rezultati koji su pripremani za štampu u *Word*-u nakon čega su potpisivani i pečatirani. Rezultati su krajem marta počeli da se unose u Kovid-19 bazu podataka, međutim nije jenjavao pritisak za izdavanjem štampanih rezultata (potvrda) za pojedinačna lica. Pored toga, zdravstvene ustanove i zavodi za javno zdravlje su zahtevali i zbirne tabele sa rezultatima za sve uzorke koje su poslali određenog dana. Takva praksa je dovodila do preteranog iscrpljivanja kadra koji je radio na izdavanju rezultata. Tokom aprila i maja Kovid-19 baza je u nekoliko navrata usavršavana pa su se tokom unošenja pojedinačnih rezultata pojedina polja automatski popunjavala, a omogućen je i unos više rezultata iz pripremljene *Excel* tabele. Tu opciju je koristila laboratorija Vatreno oko Beograd od početka rada u aprilu, Torlak od juna, a u ostalim laboratorijama koje sam obišao te pogodnosti nisu korišćene.

Kovid-19 baza je bitno evoluirala od marta do danas, ali još uvek postoji dosta prostora za unapređenje. Pretraživanje baze je omogućeno po određenom broju kriterijuma koji su vezani za lice, ali nikako nije omogućeno pretraživanje i filtriranje po svim poljima koja se popunjavaju u bazi ili po unetim vrednostima i nisu ispunjeni FAIR principi (*findability, accessibility, interoperability and reusability*) (3). Jasno je da nije bilo vremena i resursa da se napravi takva baza podataka, ali je to nešto čemu treba da se stremi. Za svaki uneti uzorak bi trebalo da postoje podaci sa kojim reagensima je obrađivan i amplifikovan i koje su bile Ct vrednosti IPC i virusnih gena. U idealnom slučaju u bazu bi se unosili i meta podaci kao što je izvorni fajl sa PCR mašine i fotografija ploče pre i nakon PCR-a na kojima bi se videle oznake eksperimenta i nivoi tečnosti i ti podaci bili bi povezani (linkovani) sa uzorkom. U takvom slučaju bi interpretacija rtRT-PCR rezultata mogla da se obavlja i izvan laboratorije. Dodatno, moglo bi da bude korisno otvaranje mogućnosti geolociranja mesta boravišta i prebivališta pacijenta, kao i mesta uzorkovanja i obrade uzoraka.

Na osnovu ovih podaka mogle bi da se dobijaju sledeće informacije: 1) koliko je osoba laboratorijski testirano; 2) koliko je osoba testirano rtRT-PCR metodom; 2a) koliko je od tih testova bilo pozitivnih (broj i procenat); 2b) koliko je od tih testova bilo prvo testiranje neke osobe; 2c) koliko je od tih testova bilo pozitivnih (broj i procenat); 3) koliko je urađeno seroloških testova; 3a) koliko je urađeno imunohromatografskih testova; 3b) koliko je urađeno enzimskih testova; 3c) kolika je seroprevalencija među testiranima tog dana; 3d) kolika je kumulativna seroprevalencija među testiranima; 4) koliko je urađeno imunohromatografskih testova na antigen. Takva struktura podataka bi mnogo jasnije potkrepila izjave zvaničnika o ozbiljnosti epidemiološke situacije.

Kadrovske potrebe za testiranje kliničkih uzoraka na SARS-CoV-2

Već u trećoj dekadi marta postalo je jasno da postojeći kadrovski kapaciteti laboratorije za testiranje na Torlaku nisu dovoljni za potrebe Srbije za dijagnostiku Kovid-19. Razlozi su brojni, od neadekvatnih materijalno tehničkih uslova do nedostatka ljudstva. Nedostatak ljudstva je delimično posledica nedovoljnog broja zaposlenih sa odgovarajućim kvalifikacijama za učešće u dijagnostici, zadržavanje određenog broja koji su raspoređeni na dijagnostiku u karantinu i nemogućnosti povlačenja zaposlenih u ostalim radnim jedinicama, takođe zbog karantina. Analiza na SARS-CoV-2 jeste deo panela mikrobioloških analiza, i kao takva trebala bi da bude pod jurisdikcijom specijalista mikrobiologije sa parazitologijom. Međutim, po svojoj suštini, sve analize koje se rade u dijagnostici Kovid-19 su ili molekularne tehnike (kao npr. rtRT-PCR) ili antigen-antitelo reakcije. Ta činjenica opravdava učešće drugih specijalista laboratorijskih grana medicine i farmacije, ali i drugih profila kojima su te tehnologije bliske i koji ih koriste u svakodnevnom radu npr. veterinari, molekularni biolozi i drugi. Potpuno je jasno da većinu testova nisu uradili i većinu rezultata nisu izdali specijalisti mikrobiologije sa parazitologijom. Moj utisak je bio da je ta činjenica ostavila gorak utisak među kolegama mikrobiolozima, ali činjenica pred kojom ne smemo zatvarati oči je da službe mikrobiologije u

in comparison to the detection with another machine, and therefore, additional analyses should be done and recommendations should be made.

Programming the PCR machines for SARS-CoV-2 testing with the rtRT-PCR method

All reagents for rtRT-PCR testing for SARS-CoV-2 had clearly written instructions for programming the temperature profiles of reaction, in the sense of defined temperatures and the number of cycles which were repeated. However, the ramp rate was not defined in the instructions, which is an important parameter of PCR and can influence the outcome of testing and the reproducibility of results. The manual entry of samples into the software, which controls the machine, is a slow process that has a great possibility of mistakes. Due to that reason, it is best to use *Excel* templates, which on the one hand facilitate the entry, and on the other communicating the results. Appropriate *Excel* templates were applied in the laboratories "Torlak" and "Fiery Eye", Belgrade. Creating the table, that is, the list for the numbers of samples was solved at the Institute "Torlak" with the autofill command in *Excel* (whenever it was possible), whereas in "Fiery Eye" the plan of a plate for the extraction of RNA was copied and previously entered into *Excel* with the barcode scanner. My impression is that in other laboratories, which I have visited, the samples were entered manually into the software during the machine programming.

The interpretation of results of rtRT-PCR tests for SARS-CoV-2

The interpretation of gained results varied significantly, which is the logical consequence of using different tests. The easiest interpretation of results was when tests with one target viral sequence (e.g. *BGI*) were used. Recommendations for the interpretation of results have changed, and in the most recent protocols, it has been recommended that the result is issued as positive when even one viral gene was positive (most frequently N gene). Discussion and exchange of experience among heads of different laboratories would be extremely useful and they would contribute to the better quality interpretation of results. Unfortunately, the practice was not established that the heads of laboratories periodically meet and discuss the interpretation of results, primarily due to the extreme efforts to maintain the scope of the necessary diagnostic, the distance between laboratories, and working in shifts.

Communicating results of rtRT-PCR tests for SARS-CoV-2

Communicating results has been challenging and it evolved from March to August. At the beginning of this epidemic, at the Institute "Torlak", results were manually written into the protocol of laboratory and then printed results were issued. They were prepared in Word, and after printing signed and stamped. At the end of March, they started to enter the results into the Covid-19 database, however, the pressure to issue printed results (findings) to individuals did not stop. In addition to that, health care institutions and public health institutes demanded collective tables with results for all samples, which they sent on that day. Such practice led to the overburdening of personnel, who issued results. During April and May, the Covid-19 database was a few times improved, so during the entry of individual results certain fields were automatically filled in, and the entry of more results from the prepared *Excel* table was made possible. That option has been used in the laboratory "Fiery Eye", Belgrade since April, at the Institute "Torlak" since June and in other laboratories, which I have visited, this convenience has not been used.

The Covid-19 database has significantly evolved since March, but still further improvements can be made. Searching the database was possible according to the criteria connected with persons, but searching and filtering by all fields in the database or by entered values were not possible, and FAIR principles were not fulfilled (*findability, accessibility, interoperability, and reusability*) (3). It is clear that there was not enough time and resources to make such a database, but it is something we should strive to. For each sample, data should be entered about reagents, with which it was processed and amplified, about Ct values and viral genes. Ideally, the meta-data would be entered into the database, such as the original file from the PCR

sekundarnoj i tercijarnoj zdravstvenoj zaštiti, kao ni po regionalnim zavodima za zaštitu zdravlja, nisu kadrovski normirane, niti je ljudstvo obučeno i spremno da iznese teret jednog ovako masovnog testiranja. Neizmeran doprinos u održavanju dijagnostike u Beogradu i drugim univerzitetskim centrima su dali zaposleni sa različitih fakulteta i naučnih instituta koji su činili okosnicu visoko kvalifikovanog ljudstva angažovanog u dijagnostici Kovid-19 i koji su radili bez ikakve naknade. Pored toga, veliki teret u dijagnostici u Šumadiji i zapadnoj Srbiji i južnoj i istočnoj Srbiji izneli su veterinarski specijalistički instituti u Kraljevu, Šapcu i Nišu koji su učestvovali u testiranju, takođe, radeći bez ikakve naknade i često trošeći sopstvene potrošne materijale.

Zaključak

Jasno je da medicinske mikrobiološke službe nisu normirane za borbu protiv pandemije ili velikih epidemija. Najefikasniji način da se prevaziđe takva situacija je da se definišu timovi koji bi se uključivali u dijagnostiku kada to epidemiološka situacija nalaže. Timovi bi trebali da budu sastavljeni po regionalnom principu i da sadrže 2-3 tehničara i 2-3 visoko kvalifikovana člana od kojih bi makar jedan član morao da bude mikrobiolog. Tehničare bi trebalo regrutovati iz raznih zdravstvenih i drugih laboratorija, a ne isključivo iz mikrobioloških laboratorija (npr. sanitarni tehničari). Visoko kvalifikovani kadar bi se regrutovao iz istraživačkih i dijagnostičkih laboratorija. Poželjno bi bilo da u svakom timu bude jedan administrativni radnik (administracija i logistika) i jedan IT stručnjak za komunikaciju i podršku. Takav tim bi mogao da uzorkuje, obradi i izda rezultate za oko dve stotine i sedamdeset osoba na jednoj standardnoj PCR mašini u šestočasovnoj smeni ili za oko sto osamdeset osoba za četiri sata, u zavisnosti od potreba. Takvi timovi ne bi iziskivali novo zapošljavanje i hipertrofisanje postojećih struktura van epidemije, a tokom epidemije bi mogli brzo da se angažuju i uključe u dijagnostiku. Bilo bi potrebno da se organizuju periodično i okupljanja članova tima, kao i da se sprovode obuke i vežbe za kriznu situaciju. Takođe, potrebno bi bilo da postoji između vođa timova periodična komunikacija van perioda epidemije, a svakodnevna tokom epidemije.

Potrebno je težiti da oprema bude unificirana na celom prostoru Republike Srbije jer se na taj način olakšava održavanje, nabavka rezervnih delova i potrošnih materijala, a verovatno može da se umanji i nabavna cena. Ovakav pristup možda nije u skladu sa slobodama tržišta, ali u kriznim situacijama bi bio izuzetno isplativ. Reagensi za ekstrakciju i specifični reagensi za rtRT-PCR (prajmeri i probe) trebali bi da budu standardizovani i domaćeg porekla kako bi se umanjila zavisnost od uvoza i značajno uštedeo novac. Pored toga, tako bi bila olakšana mobilnost kadra između laboratorija, olakšana i uniformisana interpretacija rezultata i merljiva efikasnost rada u laboratorijama.

Baza podataka bi trebalo da bude unapređena i organizovana na FAIR principima kako bi se maksimalno iskoristila mogućnost izvođenja širih zaključaka.

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machine and the photograph of the plate before and after PCR, which would show the sign of the experiment and the level of liquid and those data would be linked to the sample. In that case, the interpretation of rtRT-PCR results could be conducted out of the laboratory. In addition to that, the possibility to locate the patient's place of residence would be useful, as well as the place of sampling and sample processing.

According to these data, the following information could be gained: 1) how many people were tested in the laboratory; 2) how many people were tested with the rtRT-PCR method; 2a) how many of these tests were positive (number and percentage); 2b) how many of these were the first tests; 2c) how many of these tests were positive (number and percentage); 3) how many serology tests were conducted; 3a) how many immunochromatographic tests were conducted; 3b) how many enzyme tests were conducted; 3c) how high the seroprevalence was; 3d) how high the cumulative seroprevalence was; 4) how many immunochromatographic tests were conducted for the antigen. Such a structure of data would clearly support the statements of officials about the seriousness of the situation.

Personnel requirements for testing of clinical samples for SARS-CoV-2

In the third decade of March, it became clear that the existing personnel capacities of the laboratory at the Institute "Torlak" were not sufficient for the needs of Serbia for the diagnostics of Covid-19. The reasons are numerous, from the inadequate material-technical conditions to the lack of personnel. The lack of personnel is partly the consequence of the insufficient number of employees with appropriate qualifications, who could participate in the diagnostics, keeping some of them in the quarantine and inability to call the employees from other departments, also due to quarantine. The analysis of SARS-CoV-2 is part of the panel of microbiological analysis, and as such, it should be under the jurisdiction of specialists of microbiology with parasitology. However, in its essence, all analyses, which are conducted in Covid-19 diagnostics, are molecular techniques (such as rtRT-PCR) or antigen-antibody reaction. That fact justifies the participation of other specialists of laboratory branches of medicine and pharmacy, or the other profiles, close to those technologies who use them in everyday work, for example, veterinarians, molecular biologists, and others. It is completely clear that the majority of tests and the majority of results were not conducted or issued by the specialists of microbiology with parasitology. My impression is that this fact was a bitter blow for microbiologists, but we should not neglect the fact that microbiological departments in the secondary and tertiary health care institutions, as well as in public health institutes, are not standardized regarding personnel, and they are not trained and prepared to take the burden of such mass testing. The immeasurable contributions to the maintenance of diagnostics in Belgrade and other university centers were made by the employees from different faculties and scientific institutes, who made the key integral part of highly-qualified personnel engaged in the diagnostics of Covid-19 and who worked with no recompense. In addition to that, a great burden of the diagnostics in the Sumadija and western Serbia, the southern and eastern Serbia was carried out by veterinarian specialist institutes in Kraljevo, Sabac, and Nis, which took part in testing, as well, working with no recompense and often spending their own consumables.

Conclusion

It is clear that medical microbiological services are not standardized for the fight against the pandemic or big epidemics. The most efficient way to overcome such a situation is to define teams, which would take part in the diagnostics when the epidemiological situation orders that. Teams should be made according to the regional principle and they should include 2-3 technicians and 2-3 highly-qualified members, and one of them should necessarily be a microbiologist. Technicians should be recruited from various health care and other laboratories, and not solely from microbiological laboratories (e.g. sanitary technicians). Highly-qualified cadre would be recruited from the research and diagnostic laboratories. It would be desirable that each team has one administrative worker (administration and logistics) and one IT professional for communication and support.

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Such a team could collect samples, process, and issue results for about two hundred and seventy people on one standard PCR machine during the shift of six hours or for about one hundred and eighty people for four hours, depending on the needs. Such teams would not demand new employment and hypertrophy of the existing structures out of the epidemic, and during epidemic, they could quickly be engaged and included in the diagnostics. It would be necessary to organize periodic meetings for team members, as well as training and practice for the crisis situation. Also, it is necessary that heads of teams communicate periodically out of the epidemic, and on a daily basis during the epidemic.

The equipment should be unified on the whole territory of the Republic of Serbia because in that way, the maintenance is made easier, as well as the procurement of spare parts and consumables, and possibly the purchasing price can be lowered. Such an approach may not be in accordance with the freedom of the market, but during the period of crisis, it would be extremely profitable. Reagents for the extraction and specific reagents for rtRT-PCR (primers and probes) should be standardized and made in Serbia so that the dependence on import would be reduced and money savings would be significant. In addition to that, the mobility of cadre between laboratories would be made easier, the interpretation of results would be uniform and easier and the efficiency of work in laboratories would be measurable.

The database should be improved and organized according to the FAIR principles so that the possibility of making wider conclusions would be maximally used.

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AKTUELNA TEMA

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ZNAČAJ BRZE DIJAGNOSTIKE SARS-CoV-2, DOBRE ZDRAVSTVENE ORGANIZACIJE I RAZUMNOG KORIŠĆENJA ZNANJA I RESURSA TOKOM BORBE PROTIV KOVID-19

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SAŽETAK

U decembru 2019. godine pojavila se nova bolest, upala pluća nepoznatog uzroka, povezana sa pijacom morskih plodova u mestu Vuhan (Kina). Na humanim epitelnim ćelijama respiratornog trakta je izolovan jedan nov koronavirus, nazvan prema kliničkoj slici „teški akutni respiratorni sindrom korona virus 2" (SARS-CoV-2), dok je bolest nazvana koronavirusna bolest 2019 (Kovid-19). Pokazalo se da je ova pandemija veliki izazov, kako za stručnjake koji se bave mikorbiološkom dijagnostikom, tako i za sve zdravstvene radnike. Cilj ovog rada je da prikaže značaj brze dijagnostike SARS-CoV-2, značaj zaštite zdravlja zdravstvenih radnika i razumnog korišćenja znanja i resursa tokom borbe protiv Kovid-19. Dosadašnja istraživanja potvrđuju da su u borbi protiv SARS-CoV-2 infekcije od posebnog značaja brza dijagnostika SARS-CoV-2, zaštita zdravlja zdravstvenih radnika i razumno korišćenje znanja i resursa tokom pandemije. Poseban akcenat se stavlja na sprovođenje vakcinisanja protiv sezonskog gripa, kao i protiv invazivne pneumokokne bolesti, jer primenom ovih vakcina možemo olakšati postavljanje dijagnoze Kovid-19 u budućem period u kome se sa velikim nadama očekuje efikasna antivirusna terapija za SARS-CoV-2 i vakcina za Kovid-19.

Ključne reči: Kovid-19, SARS-CoV-2, mikrobiološka dijagnostika, zdravstveni radnici, znanje, resursi

Uvod

Grupa pacijenata sa pneumonijom nepoznatog uzroka povezana je sa pijacom morskih plodova u Vuhanu, u Kini, u decembru 2019. godine. Iz epitelnih ćelija respiratornog trakta čoveka izolovan je novi koronavirus, koji je nazvan prema kliničkoj slici „teški akutni respiratorni sindrom korona virus 2" (SARS-CoV-2). U februaru 2020. godine virus je dobio zvanično ime teški akutni respiratorni sindrom korona virus 2 (engl. *Severe Acute Respiratory Syndrome Corona Virus 2* – SARS-CoV-2). Bolest je dobila ime koronavirusna bolest 2019 (Kovid-19) (1). Procenjeno je da SARS-CoV-2 ima reproduktivni broj (R_0) 2 – 2,5 u prvoj fazi nekontroliranog širenja bolesti, što znači da će se 2 do 3 osobe zaraziti od nultog pacijenta. Grip ima R_0 broj 1,2 (1,2). U prvoj nedelji aprila prijavljen je 1 milion potvrđenih slučajeva i 50.000 smrtnih ishoda (3,4). SARS-CoV-2 virus je izolovan i kod asimptomatskih slučajeva, sa druge strane, neki inficirani pacijenti nastavljaju da budu pozitivni na testu reverzne transkripcije lančane reakcije polimeraze (engl. *Reverse Transcription Polymerase Chain Reaction* – RT-PCR) dve do tri nedelje nakon pojave simptoma (1-3).

Pokazalo se da je Kovid-19 naročito smrtonosna bolest kod starijih pacijenata. U prvoj velikoj studiji iz Vuhana, 137 pacijenata je otpušteno iz bolnice, a 54 je umrlo u bolnici. Većina umrlih pacijenata činili su stariji pacijenti sa komorbiditetima. Pre-življavanje pacijenata kojima je bila potrebna oksigenoterapija takođe je bilo nisko. Preživelo je 20% pacijenata koji su bili na oksigenoterapiji koja je uključivala nazalnu kanilu sa visokim protokom kiseonika u odnosu na 8% preživelih na neinvazivnoj mehaničkoj ventilaciji i 3% preživelih na invazivnoj mehaničkoj ventilaciji (5). SARS-

ACTUAL TOPIC

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THE SIGNIFICANCE OF FAST DIAGNOSTICS OF SARS-COV-2, GOOD HEALTH CARE ORGANIZATION AND REASONABLE USE OF KNOWLEDGE AND RESOURCES IN THE FIGHT AGAINST COVID-19

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SUMMARY

In December 2019, a new disease appeared, that is, pneumonia of unknown cause, which was linked to a seafood market in Wuhan (China). Human respiratory epithelial cells were used to isolate a novel coronavirus, named according to the clinical picture "Severe Acute Respiratory Syndrome Coronavirus 2" (SARS-CoV-2), whereas the disease was named the coronavirus disease 2019 (Covid-19). This pandemic has proved to be a great challenge for all professionals dealing with microbiological diagnostics, as well as for all health care workers. The aim of this work is to present the significance of fast diagnostics of SARS-CoV-2, the significance of protection of health of health care workers, and the reasonable use of knowledge and resources in the fight against Covid-19. The previous research has confirmed that in the fight against the SARS-Cov-2 infection, fast diagnostics of SARS-CoV-2, protection of health of health care workers and reasonable use of knowledge and resources during the pandemic are of utmost importance. A special emphasis is put on the vaccination against seasonal influenza, as well as against the invasive pneumococcal disease because, with the help of these vaccines, the diagnosis of Covid-19 could be made easier in the future period, in which the efficient antiviral therapy for SARS-CoV-2 and the vaccine for Covid-19 are expected with great hopes.

Key words: Covid-19, SARS-CoV-2, microbiological diagnostics, health care workers, knowledge, resources

Introduction to the pandemic of Covid–19

In December 2019, a cluster of patients with pneumonia of unknown cause was linked to a seafood wholesale market in Wuhan, China. Human airway epithelial cells were used to isolate a novel coronavirus, named according to the clinical picture "severe acute respiratory syndrome 2" (SARS-CoV-2). The virus got an official name in February 2020, Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2). The disease was named coronavirus disease 2019 (COVID-19) (1). SARS-COV-2 has an estimated reproduction number (R0) of 2– 2.5 in the first phase of the uncontrolled spreading of the disease indicating that 2 – 3persons will be infected from an index patient. Influenza has R0 of 1.2 (1,2). In the first week of April 1 million confirmed cases, with more than 50,000 deaths were reported (3,4). The SARS-CoV-2 virus has been isolated from asymptomatic individuals, and affected patients continue to be positive on the reverse transcriptase polymerase chain reaction (RT-PCR) test two to three weeks after the start of symptoms (1-3).

COVID-19 has proved to be particularly deadly in elderly patients. In the first major study from Wuhan, 137 patients were discharged from the hospital, while 54 died in the hospital. The vast majority of those who died were the elderly with comorbidities. The survival of patients that needed oxygen support was also bad. 20% of patients on high-flow nasal cannula oxygen therapy survived in comparison to 8% of patients on non-invasive mechanical ventilation and 3% of patients on invasive mechanical ventilation (5). SARS-CoV-2 hit particularly hard

CoV-2 je posebno teško pogodio evropske mediteranske zemlje i njihove starije stanovnike (4). Najteže pogođena regija je bila Lombardija, a većina pacijenata koji su preminuli imali su pridružene bolesti (6).

Pacijenti kod kojih je imunitet kompromitovan predstavljaju grupu pacijenata koji su najviše ugroženi kada je u pitanju Kovid-19. Primećeno je da oboleli od kancera imaju veći rizik od težih formi Kovid-19 bolesti (češće se primaju na odeljenje intenzivne nege i zahtevaju invazivnu ventilaciju, ili kod njih dolazi do smrtnog ishoda) u poređenju sa pacijentima koji nemaju kompromitovan imuni sistem (7-9). „Tri musketara" u borbi protiv SARS-CoV-2 infekcije su brza dijagnostika SARS-CoV-2, zaštita zdravlja zdravstvenih radnika i razumno korišćenje znanja i resursa tokom pandemije (10,11).

Cilj ovog rada je da prikaže značaj brze dijagnostike Kovid-19 bolesti, čuvanja zdravstvenog stanja zdravstvenih radnika i razumnog korišćenja znanja i resursa tokom borbe protiv Kovid-19

Dijagnostika Kovid-19

Trenutno se dijagnostika Kovid-19 bazira na primeni RT-PCR testa. Prvi set opreme je razvijen ubrzo nakon otkrića virusa. U partnerstvu Nemačke i Hong Konga objavljen je članak o razvoju RT-PCR testa (februar, 2020) koji je detektovao i amplifikovao dva različita gena: E (proteinski omotač) i RdRp (RNA-zavisna RNA polimeraza) (5).

Drugi RT-PCR test su dizajnirali Centri za kontrolu i preenciju bolesti Sjedinjenih Američkih Država (engl. *Centers for Disease Control and Prevention* - CDC), koji je mogao da detektuje tri različita gena: područje NS3 je univerzalno za detekciju korona virusa sličnih SARS-u, a N1 i N2 područja su specifična samo za SARS-CoV-2. Testovi su validirani po pitanju svih karakteristika tačnosti (uključujući senzitivnost i specifičnost). Sa trajanjem pandemije brojne kompanije razvijaju i komercijalizuju RT-PCR testove za SARS-CoV-2.

Vreme uzimanja uzorka za analizu se pokazalo kao veoma značajno. Generalno, količina respiratornih virusa je najviša dva dana nakon početka simptoma, te se stoga preporučuje uzimanje uzorka što je pre moguće od početka simptoma. Odlaganje uzimanja uzoraka može da rezultira lažnim negativnim rezultatima, što je generalno karakteristika i ostalih respiratornih virusa. Sposobnost RT-PCR testa da detektuje korona virus u velikoj meri zavisi od kvaliteta uzorka. Ako se nazofaringealni bris (NFB) ne ubaci dovoljno duboko i pravilno u nazofaringealni prostor, odnosno ako se ubaci samo u nozdrve, vrlo je verovatno da će rezultat biti lažno negativan kod pacijenta inficiranog SARS-CoV-2. Prvilno uzimanje uzorka je neophodno, jer ako uzorak nije uzet iz dela gde se virus nalazi, virus neće biti otkriven, bez obzira koliko je RT-PCR test kvalitetan (10). Kod pacijenata sa pneumonijom, savetuje se uzimanje uzorka iz donjeg respiratornog trakta, ali je problem invazivnost metode. Bilo je slučajeva kada je NFB bio negativan, ali je bronhoalveolarna lavaža (BAL) dala uzorak koji je bio pozitivan na RT-PCR testu za SARS-CoV-2 (12).

Postoje problemi i u vezi izvođenja RT-PCR testa. Potrebno je da ga radi veoma iskusno osoblje i zahteva dosta vremena. Takođe, zbog visoke senzitivnosti može da detektuje i kopije gena mrtvog virusa SARS-CoV-2. Evropski centar za prevenciju i kontrolu bolesti (engl. *European Centre for Disease Prevention and Control* - ECDC) savetovao je dodatnu proveru za visoke granične vrednosti (na primer Ct vrednost, engl. *Cycle threshold value*, > 35) RT-PCR testa. Ovaj Centar predlaže da se pozitivni rezultati testa koji imaju visoke Ct vrednosti ponovo razmotre u sklopu osnove kliničke slike (13).

Do sada se zna da je osoba veoma zarazna sve do trenutka kada virus nije moguće izolovati iz ćelijskih kultura. Širenje respiratornog virusa je bilo vrlo visoko tokom prve nedelje simptoma u jednoj nemačkoj studiji (14). Iako je velika količina virusnih RNA dobijana i nakon prestanka simptoma, serokonverzija se javljala nakon 7 dana kod 50% pacijenata. Nijedan virus nije izolovan u ćelijskim kulturama nakon sedmog dana (14,15). I dalje treba uzeti u obzir da jedan negativan test na SARS-CoV-2 (posebno ako je uzorak uzet iz gornjeg respiratornog trakta) ili pozitivan rezultat testa na neki drugi respiratorni patogen, ne isključuju infekciju SARS-CoV-2 (13,16). Ako je prvi RT-PCR test negativan, to ne znači da nije potrebno drugo uzimanje uzorka. Ako pacijent dobije tempe-

the European Mediterranean countries and their elderly inhabitants (4). The area hardest hit by the virus was the region of Lombardy, while the majority of patients in Lombardy who died had the underlying health conditions (6).

Immunocompromised patients are the group of people that are most endangered due to their suppressed immune status when it comes to Covid-19. Patients with cancer were observed to have a higher risk of severe forms of the Covid-19 disease (they are admitted to the intensive care unit more frequently, requiring invasive ventilation, or it comes to deathly outcomes) compared with the patients without the suppressed immune system (7,9). "The three musketeers" of the fight against the SARS CoV-2 infection are the following: fast diagnostics of SARS CoV-2, protection of health of health care workers, and reasonable use of knowledge and resources in the SARS CoV-2 pandemic (10,11).

The aim of this work is to present the significance of fast diagnostics of the Covid-19 disease, protection of health of health care workers, and reasonable use of knowledge and resources during the struggle against Covid-19.

Diagnostics of Covid-19

Current diagnostics is based on RT-PCR test (Reverse transcription polymerase chain reaction). The first kit was developed very soon after the discovery of the virus. The Germany-Hong Kong partnership published the article about the development of the RT-PCR test (February 2020), which detected and amplified two different genes: the E (envelope protein) and RdRp (RNA-dependent RNA polymerase) genes (5).

The United States Centres for Disease Control and Prevention (CDC) designed the other RT-PCR test, which could detect three different genes: the NS3 region for the universal detection of SARS-like coronaviruses, and the N1 and N2 regions, which are specific for SARS-CoV-2. Assays have been validated for all characteristics of precision (including sensitivity and specificity). With the continuation of this pandemic, numerous companies have developed and commercialized RT-PCR tests for SARS-CoV-2.

The time of the collection of samples has proved to be very important. Generally, respiratory viral loads peak two days after the onset of the symptoms, and therefore, the samples are recommended to be taken as soon as possible. Delays in the collection of samples can result in false-negative results, and this is generally true for other respiratory viruses, as well. The ability of the RT-PCR test to detect coronavirus will largely depend on the quality of the sample. If a nasopharyngeal (NP) swab is not inserted deeply and properly into the nasopharyngeal space, that is, if it is inserted only into the nostrils, it is likely that this will result in a false negative result in the patient infected with SARS-CoV-2. The proper collection of samples is needed because if the sample hasn't been collected from an area, where the virus is likely to be, the virus will not be detected regardless of how good the RT-PCR test is (10). In patients with pneumonia, it is advisable to collect the specimen from the lower respiratory tract, but this method can be problematic because it is invasive. We have seen cases when NPS was negative, but the bronchoalveolar lavage (BAL) yielded the sample that was positive for SARS-CoV-2 on the RT-PCR test (12).

There are some problems regarding the RT-PCR test. You need very experienced personnel to do it and is time-consuming. Also, due to its sensitivity, it can detect the copies of genes of dead SARS-CoV-2. The European Centre for Disease Control (ECDC) advised the additional control of high RT-PCR cycle threshold values (e.g. Ct > 35). This Centre suggested that positive test results that have high Ct values should be reconsidered according to the clinical picture (13).

By now we know that person is very contagious till the moment when we are not able to isolate the virus in cell cultures. The respiratory virus shedding was very high during the first week of symptoms in a German study (14). Although shedding a large amount of viral RNA outlasted the end of symptoms, seroconversion occurred after 7 days in 50% of patients. No viruses were isolated in cell cultures after day 7 (14,15). We still have to take into consideration that a single negative SARS-CoV-2 test (especially if from an upper respiratory tract specimen) or a positive test result for another respiratory pathogen does not exclude the SARS-CoV-2 infection (13,16). If the first RT-PCR is negative that does not mean

raturu ili ima respiratorne tegobe, savetuje se ponovno uzimanje drugog uzorka iz donjeg dela respiratornog trakta. Ako je test negativan, treba tražiti druge razloge pogoršanja (11,13). Značan napredak u dijagnozi Kovid-19 infekcije je postignut automatizacijom laboratorijskog procesa. Ovakve sisteme su razvili *Cobas*® *Liat*® (*Roche Molecular 145 Systems, USA*) i *GeneXpert*® (*Cepheid, USA*). Njihova prednost je što se klinički uzorak u medijumu za transport virusa prebacuje u kertridž aparata i što se izvode u laboratorijama biološke bezbednosti klase II/III. Ovi sistemi integrišu izdvajanje virusne nukleinske kiseline (NK), amplifikaciju NK i detekciju specifičnih delova genoma SARS-CoV-2. Osoblje koje radi ove analize treba da ima propisanu zaštitnu opremu (13,16)

SARS-CoV-2 je veoma nepredvidljiv, pa neki pacijenti imaju nespecifične simptome zbog toga je diferencijalna dijagnostika značajna, a trebalo bi da uključi druge respiratorne viruse, *Pneumocystis jirovecii* i atipične bakterije kao što je *Legionella pneumophila*. Zato je ključno da se rezultat RT-PCR na SARS-CoV-2 brzo dobije (slika 1), idealno još tokom pregleda pacijenta (16,18). Postoje dva testa koja daju rezultate za manje od 30 minuta: *ID Now* (*Abbot, USA*) i *Accula SARS-CoV-2 test* (*Mesa Biotech, USA*) (18). Ovi testovi su brzi zato što je korak denaturacije deo procesa i sve se sprovodi na jednoj temperaturi. Oba testa koriste izotermalnu amplifikaciju, i stoga nisu senzitivni kao RT-PCR, a problem može da nastane ako je uzorak krvav ili ako na njemu ima mnogo sluzi. Postoje i brzi antigenski testovi. Jedan od njih je SARS *Sofia* test (*Quidel, USA*). Ovaj test se bazira na flourescentnoj detekciji veze protein – antigena za monoklonska antitela. Za analizu testa je potreban analizator. Za 15 minuta mogu se analizirati dva uzorka. Uzorak je suvi bris NZF. Test je nešto manje senzitivan nego RT-PCR, a treba ga raditi samo kod pacijenata sa simptomima. Sve brze testove je potrebno klinički evaluirati (13).

Perspektive dijagnoze Kovid-19 u sezoni gripa

Budućnost brze i tačne dijagnoze Kovid-19 u sezoni gripa će biti istovremeni „*multiplex*" PCR skrining na veći broj respiratornih patogena, posebno kod dece i imunokompromitovanih pacijenata. Takođe, u nastupajućoj sezoni gripa brza dijagnostika gripa i SARS-CoV-2, na mestu pružanja nege pacijentu, biće ključni korak za smanjenje nepotrebnog korišćenja antibiotika (16,17). Jedno od budućih rešenja jeste i kreiranje višestrukog PCR testa koji može da detektuje sva četiri korona virusa (18,19).

Zdravstveni radnici: njihova uloga i zaštita tokom pandemije Kovid-19

U vreme pandemije SARS-CoV-2, najvažnije je da zdravstveni radnici ne šire virus u populaciji osetljivih ljudi, a posebno na imunokompromitovane pacijente (7,9). Zdravstveni radnici mogu da dobiju virus od pacijenata sa i bez simptoma ako nisu adekvatno zaštićeni. Takođe, SARS-CoV-2 se može preneti sa plastičnih površina i nerđajućeg čelika. Živ virus je otkriven 72 sata nakon kontaminacije ovih površina (20).

Kovid-19 infekcija kod zdravstvenih radnika može biti asimptomatska ili simptomatska (od lake do kritične). Veliki problem predstavlja kada lekari ne znaju svoj status, kako po pitanju Kovida-19, tako i drugih zaraznih bolesti. Ako se zdravstveni radnik inficira ili je bio u visoko rizičnom kontaktu, trebalo bi postaviti standarde kada on može da se vrati na posao. Ako je osoba bila u kontaktu visokog rizika treba da bude u kućnoj izolaciji 14 dana. Ako zdravstveni radnik poseduje specijalna znanja i treba da radi sa svom potrebnom ličnom zaštitnom opremom, onda treba pratiti da li će se kod njega pojaviti simptomi Kovid-19. U slučaju pojave simptoma, treba ga izolovati ili hospitalizovati.

Ako se osoba zarazi, nekoliko zemalja je uvelo standard kada ta osoba može da se vrati na posao. Postoji saglasnost da simptomi treba da nestanu i da, ako ne možemo čekati 14 dana, barem jedan RT-PCR test treba da bude negativan. Italija je takođe postavila standard da je potrebna pojava imunoglobulina G (IgG) da bi se potvrdio status izlečenja (13). IgG serokonverzija je racionalna s obzirom da ako se pojave IgG antitela manje je verovatno da je osoba zarazna (21-23). Serološko testiranje bi moglo biti važno za zdravstvene radnike. Negativni serološki testovi kod zdravih pojedinaca imaju dobru negativnu prediktivnu vrednost. Pozitivan IgG znači da se zdravstveni radnik susreo sa infekcijom SARS-CoV-2 i da je

the second sampling is not necessary. If the patient has a fever or a respiratory discomfort that second sampling is advised from the lower respiratory tract. If the test is negative, we should look for other reasons for deterioration (11-13). The significant advance in diagnostics of Covid-19 was achieved by the automation of the laboratory process. Such systems were developed by are *Cobas® Liat®* (*Roche Molecular 145 Systems, USA*) and *GeneXpert®* (*Cepheid, USA*). Their advantage is that the clinical specimen in the viral transport medium is transferred into a cartridge, in class II/III biosafety laboratories. These systems integrate the separation of viral nucleic acid (NA), the amplification of NA, and the detection of specific genome parts of SARS-CoV-2. Personnel that does these analyses should wear the necessary personal protective equipment (13-16).

SARS CoV-2 is a great pretender, and some patients have non-specific symptoms. Therefore, differential diagnosis is important and it should include other respiratory viruses, *Pneumocystis jirovecii* and atypical bacteria including *Legionella pneumophila* (15) Therefore, the key thing is to get the result of the RT-PCR test for SARS-CoV-2 fast (Figure 1), ideally while examining the patient (16-18). There are two tests that promise the results in less than 30 minutes: *ID Now* (*Abbot, USA*) and *Accula* SARS-CoV-2 test (*Mesa Biotech, USA*) (18). These tests are fast because the denaturation step is part of the process and everything is run on one temperature. Both tests use the isothermal amplification, and therefore, they are not as sensitive as RT-PCR, while problems can arise if the sample is bloody or there is a lot of mucus on it. There are rapid antigen tests, as well. One of them is the *SARS Sofia* test (*Quidel, USA*). This test is based on the fluorescent detection of bond protein-antigen for monoclonal antibodies. The analyzer is necessary for the test analysis. Two samples can be analyzed for 15 minutes. The sample is the NFS. The test is less sensitive than RT-PCR, and it should be done only in patients with symptoms. All rapid tests should be clinically validated.

The perspective of Covid-19 diagnostics in the flu season

The future of the fast and precise diagnostics of Covid-19 during the flu season will be the simultaneous "multiplex" PCR screening for respiratory pathogens, especially in children and immunocompromised patients. Also, during the next flu season, the rapid influenza diagnosis with the rapid point-of-care SARS-CoV-2 diagnosis will be a crucial step that will reduce the unnecessary use of antibiotics (16,17). One of the possible solutions will be the development of the multiplex PCR test, which could detect all four coronaviruses (18,19).

Health care workers: their role and protection during the Covid-19 pandemic

In times of pandemic of SARS CoV-2, the biggest concern of the public is that health care providers do not spread the virus in the population of vulnerable people, especially immunosuppressed patients (7-9). Health care workers can get the virus from patients with or without symptoms if they are not protected properly. Also, SARS-CoV-2 can be transmitted from plastic surfaces and stainless steel. Viable virus was detected up to 72 hours after the contamination of these surfaces (20).

Covid-19 infection in health care workers can be asymptomatic or symptomatic (from mild to critical). Not knowing their status is troublesome in terms of Covid-19 and other infectious diseases. If a health care worker gets infected or had a high-risk contact, we should set standards when he can return to work. If a person had a high-risk contact, he should be in isolation at home for 14 days. If a health care worker has special knowledge, he or she should work protected with all the necessary personal protective equipment and should carefully watch for the symptoms of Covid-19. If symptoms appear, he should be isolated or hospitalized.

If a person is infected, several countries introduced standards when he or she can return to work. It is agreed that the symptoms have to disappear and that at least one PCR has to be negative if we cannot wait 14 days. Italy has also set a standard that the appearance of immunoglobulin G (IgG) is necessary for the confirmation of healed status (13). The standard

zaštićen od dobijanja bolesti bar neki vremenski period. Takvi zdravstveni radnici bi mogli da rade sa pacijentima znajući da se neće razboleti ako ponovo budu izloženi infekciji SARS-CoV-2 i da neće širiti virus. Na slici 2 dat je predlog kako treba da se zaštite zdravstveni radnici tokom pandemije (13).

Razumno korišćenje resursa i znanja tokom pandemije SARS-CoV-2

Etički principi bi takođe trebalo da budu prioritet tokom nege pacijenata u pandemiji SARS-CoV-2. Najvažniji princip je spašavanje života. Međutim, spašavanje života može biti još važnije u uslovima pandemije kada resurse treba mudro koristiti. Razvijanje dodatnih vodiča treba da bude transparentno i inkluzivno i u skladu sa najnovijim saznanjima. Štaviše, diskusija i planiranje su ključni zato što dublja razmatranja nisu izvodljiva tokom javnozdravstvene krize (22). Ako se računa na spašavanje što više života, etično je lečiti nekoga u jedinici Kovid-19 intenzivne nege ko ne želi da prihvati hemoterapiju. Međutim, tu

DIJAGNOZA: PNEUMONIJA?

Oboleli pacijenti sa temperaturom ili drugim siptomima respiratornog trakta (npr. kašalj, kratak dah, hipoksija) su prioritet za testiranje na SARS-CoV-2.

Identifikujte grip, bakterijsku pneumoniju, ili prateće pojave povezane sa lečenjem kao što su post-hirurški plućni problemi ili oni povezani sa sistemskom terapijom (npr. atelektaza, embolija, pneumonitis, edem pluća/višak tečnosti, itd.)

PRIJEM U BOLNICU

Uzmite nazofaringealni bris da biste osobu testirali na SARS-CoV-2 i druge potencijalne respiratorne patogene. Ne zaboravite da uradite krvnu sliku i testove na bakterije npr. *Legionella* i *Streptococcus pneumoniae* antigen iz urina.

Dvostruka infekcija je retka! Moguća je bakterijska superinfekcija.

TEST IZBORA

PRVI IZBOR: Brzi direktni SARS-CoV-2 PCR bez izolacije RNA ili „*multiplex*" PCR za pacijente koji će biti primljeni u jedinicu intezivne nege (respiratorni virusi + SARS-CoV-2 + atipične bakterije).

Prvi izbor za pacijente koji će biti primljeni u bolnicu (samo pacijenti sa simptomima): Ag test na SARS-CoV-2 i influenca tip A i B.

IgG testovi za antitela su adekvatni samo za pacijente koji su imali bolest sličnu Kovid-19 barem 14 dana ranije i voleli bi da provere SARS-CoV-2 status.

Ako je pacijent i dalje sumnjiv na Kovid-19 ili je dobio simptome nalik Kovid-19 tokom hospitalizacije, uradite RT-PCR koji detektuje više od 2 gena iz nazofaringealnog brisa i iz sputuma ili bronhoalveolarnog lavata (BAL-a).

PREDNOSTI I MANE

PREDNOSTI: Brzi direktni PCR/Ag test je adekvatan za pacijente sa jasnim simptomima Kovid-19, a rezultati se dobijaju za manje od 1h, ili „*multiplex*" PCR za 15 minuta. Ovi testovi ne zahtevaju mnogo osoblja i skupu opremu.

MANE: RT-PCR zahteva vreme, direktni RT-PCR/Ag test nije adekvatan za pacijente koji su ozbiljno imunokompromitovani s obzirom da mala količina SARS-CoV-2 može stvarati velike probleme – SARS-CoV-2 pneumoniju.

Slika 1. Dijagnostički algoritam za respiratorne infekcije tokom Kovid-19 pandemije (Evropski centar za prevenciju i kontrolu bolesti) (27)

of IgG seroconversion is rational since if the IgG appears the person is less likely contagious (21-23). Serology testing could be important for health care workers. Negative serology tests in healthy persons have a good negative predictive value. Positive IgG means that a health care worker met with the SARS-CoV-2 infection and that he is protected from getting infected at least for some time. Such health care workers could work with patients knowing that if they were exposed to SARS-CoV-2 again, they would not get ill and would not spread the virus around. The proposed algorithm on how to protect the health care workers during the pandemic is presented in Figure 2 (13).

Reasonable use of resources and knowledge in the SARS-CoV-2 pandemic

Ethical principles should also be our priority while taking care of patients during the pandemic of SARS-CoV-2. The most important principle is saving lives. However, saving lives can be even more important in the conditions of the pandemic when resources should be wisely

DIAGNOSIS: PNEUMONIA?

Patients with fever or other respiratory tract symptoms (eg, cough, shortness of breath, hypoxia) are a priority for testing for SARS-CoV-2.

Identify influenza, bacterial pneumonia, or treatment-related events such as post-surgical lung problems or those associated with systemic therapy (eg atelectasis, embolism, pneumonitis, pulmonary edema/excess fluid, etc.)

HOSPITAL ADMISSION

Take a nasopharyngeal swab to test the person for SARS-CoV-2 and other potential respiratory pathogens. Don't forget to do a blood test and tests for bacteria e.g. *Legionella* and *Streptococcus pneumoniae* antigen from urine.

Double infection is rare! Bacterial superinfection is possible.

TEST OF CHOICE

FIRST CHOICE: Rapid direct SARS-CoV-2 PCR without RNA isolation or multiplex PCR for patients to be admitted to the intensive care unit (respiratory viruses + SARS-CoV-2 + atypical bacteria).

The first choice for patients to be admitted to the hospital (patients with symptoms only): Ag test for SARS-CoV-2 and influenza types A and B.

IgG antibody tests are only adequate for patients who have had a disease similar to Covid-19 at least 14 days earlier and would like to check their SARS-CoV-2 status.

If the patient is still suspected of Covid-19 or has developed Covid-19-like symptoms during hospitalization, perform RT-PCR that detects more than 2 genes from nasopharyngeal swabs and from sputum or bronchoalveolar lavage (BAL)

ADVANTAGES AND DISADVANTAGES

ADVANTAGES: Rapid direct PCR/Ag test is adequate for patients with clear symptoms of Covid-19, and results are obtained in less than 1 hour, or multiplex PCR in 15 minutes. These tests do not require a lot of staff and expensive equipment.

DISADVANTAGES: RT-PCR requires time, direct RT-PCR / Ag test is not adequate for patients who are severely immunocompromised since a small amount of SARS-CoV-2 can create major problems - SARS-CoV-2 pneumonia.

FIgure 1. Diagnostic algorithm for respiratory infections during the Covid-19 pandemic (European Center for Disease Prevention and Control) (27)

Lična zaštitna oprema	Zdravstveni radnik treba da nosi FFP2=N95 maske. One jedino mogu da zaštite da se ne udahne SARS-CoV-2. Dekontaminacija maski nije dokazana u praksi. Ostala zaštitna oprema poput vizira ili naočara i hirurških odela može ponovo da se koristi ako se adekvatno primene mere dekontaminacije.
Visoko rizični kontakt sa osobom koja je pozitivna na Kovid-19	Zdravstveni radnici bi trebalo da ostanu kod kuće 14 dana ili da rade administrativni posao. Ako pokažu znake bolesti slične Kovid-19, treba uzeti uzorak za RT-PCR. Zdravstveni radnici sa specijalnim veštinama mogu da rade sa pacijentima sa FFP2 maskom. Trebalo bi da budu testirani RT-PCR metodom jednom na dva dana ako se zaraze SARS-CoV-2.
Zdravstveni radnik pozitivan na SARS-CoV-2	Treba da bude u izolaciji kod kuće, ili bolje u samoizolaciji, u odvojenom objektu. Kada se radnik oseća dobro i nema temperaturu 2 ili 3 dana može se vratiti na posao. RT-PCR i serologija se radi samo tada, kada se zaposleni vraća na posao ranije nego za 10 dana i ima još simptome Kovid-19. Nalaz IgG za „*spike*" proteina SARS-CoV-2 kod takvog radnika znači da ne može više zaraziti druge osobe.
Zdravlje i održavanje kondicije	Zdravstveni radnik treba da održava kondiciju. Postarajte se da ima svakodnevni kontakt sa porodicom, da spava mnogo i da može da se osloni na podršku kolega. Ne predlažite samoizolaciju radnicima koji su zdravi.
Održavanje pozitivnog duha	Budite kreativni. Zdrava hrana je obavezna. Uključite radnike u proces pripremanja hrane. Ponudite im časove fitnesa unutar ili blizu objekta.
Vakcinacija	Vakcinišite sve koje možete protiv influence tip A i B. Prezentujte im koristi ako se vakcinišu. Ukoliko vakcina za SARS-CoV-2 bude dostupna, organizujte vakcinaciju za sve.

Slika 2. Briga o zdravlju zdravstvenih radnika tokom pandemije Kovid-19 (13)

su i brojna pitanja na koje je teško dati odgovor. Ako ustanova nema dovoljno respiratora za sve Kovid-19 pacijente, da li respirator dati mlađem ili starijem pacijentu sa brojnim komorbiditetima i velikom verovatnoćom za letalni ishod (10,24-26).

Budućnost SARS-CoV-2

SARS-CoV-2 će ostati sa nama kao i četiri korona virusa koji koji u našoj populaciju najviše dominiraju tokom jeseni i proleća. Zato je veoma važno sprovoditi vakcinisanje protiv sezonskog gripa, kao i protiv invazivne pneumokokne bolesti. Uz ove dve vakcine možemo olakšati postavljanje dijagnoze Kovid-19 u budućem periodu u kome se sa velikim nadama očekuje efikasna antivirusna terapija za SARS-CoV-2 i vakcina za Kovid-19.

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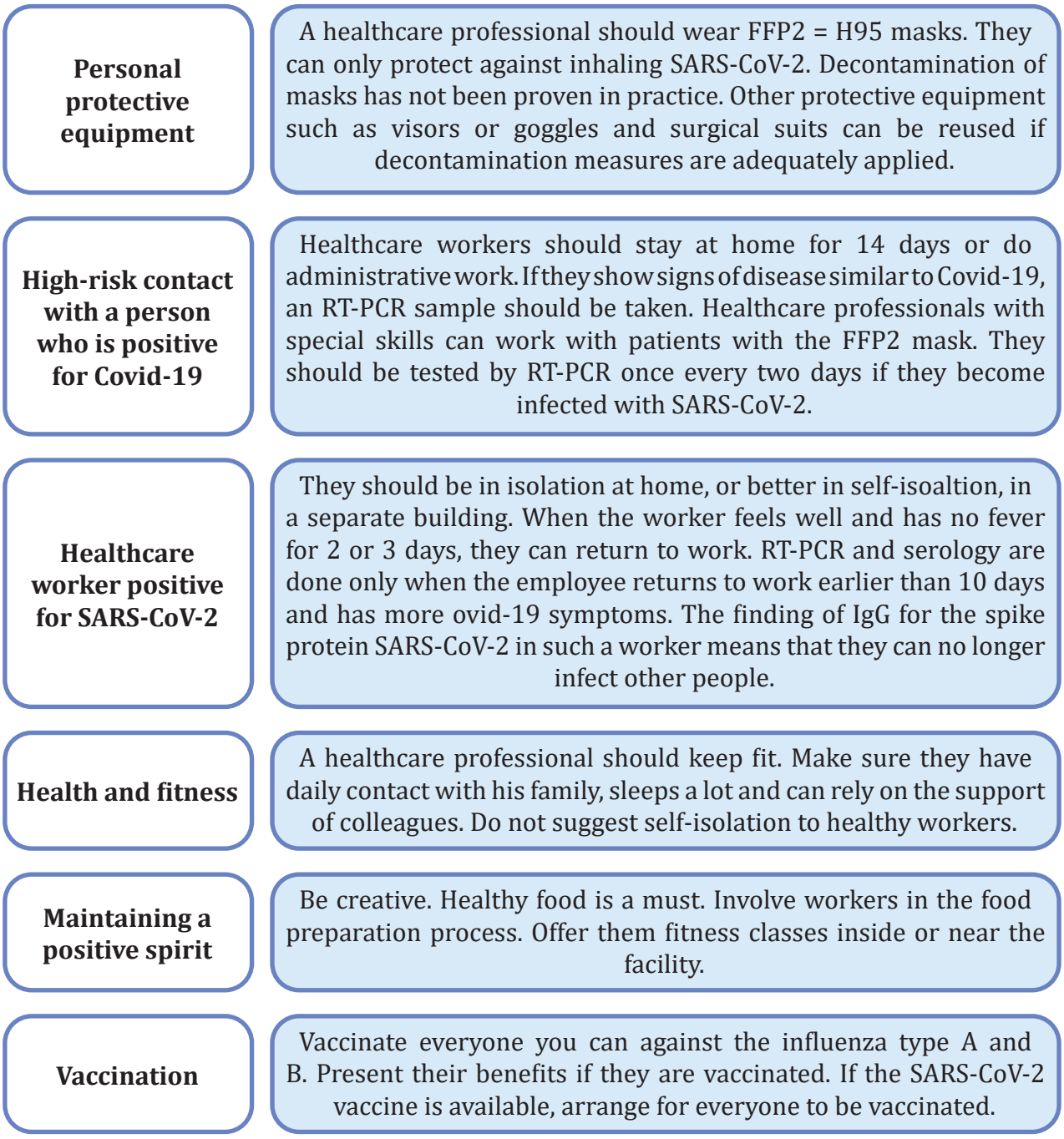

Figure 2. Health care of health workers during the Kovid-19 pandemic (13)

used. Developing additional guides has to be transparent and inclusive, and in accordance with the most recent knowledge. Moreover, discussion and planning are essential because in-depth deliberations are not feasible during a public health crisis (22). If saving the most life counts, is it ethical that we treat somebody in the intensive care Covid-19 unit who does not want to accept chemotherapy. However, there are numerous questions that are difficult to be answered to. If one institution does not have enough ventilators for all Covid-19 patients, should the ventilator be given to a younger patient or the older one with numerous comorbidities and greater possibility for the lethal outcome (10,24-26).

The future of SARS-CoV-2

SARS CoV-2 will stay with us as the other four coronaviruses tthat dominate our population the most during autumn and spring. Therefore, it is very important to conduct vaccination against seasonal influenza, as well as against invasive pneumococcal disease. With these two vaccinations, we can make the diagnosis of Covid-19 easier in the future period, in which

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the efficient antiviral therapy for SARS-CoV-2 and the vaccine for Covid-19 are expected with great hopes.

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DA LI ĆE KOVID-19 PANDEMIJA NEŠTO PROMENITI? – POGLED IZ UGLA MIKROBIOLOŠKE STRUKE

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SAŽETAK

Pandemija Kovid-19 je uzdrmala većinu zdravstvenih sistema širom sveta i pokazala da je neophodno uložiti sredstva u savremenu opremu za molekularnu dijagnostiku, kao i obezbediti adekvatan prostor u mikrobiološkim laboratorijama za ovaj vid dijagnostike, po normativima koji su za to predviđeni. Takođe, potrebno je povećati broj specijalista medicinske mikrobiologije, naročito u laboratorijama gde je nedostatak kadra alarmantan. Ulaganje u dijagnostičke grane medicine (gde spada i medicinska mikrobiologija) obezbeđuje optimalnu zdravstvenu zaštitu: brzo postavljanje tačne dijagnoze, primenu adekvatne antimikrobne terapije u kraćem roku, što vodi većoj šansi za izlečenje, smanjenju broja bolničkih dana i broju smrtnih ishoda, a ambulantnim pacijentima obezbeđuje se brže postavljanje dijagnoze i manji broj raznih, nekada nepotrebnih specijalističkih pregleda. U toku pandemije Kovid-19, Sekcija mikrobiologa Srpskog lekarskog društva, kao i Institut za mikrobiologiju i imunologiju Medicinskog fakulteta Univerziteta u Beogradu, stavili su se na raspolaganje Ministarstvu zdravlja Republike Srbije u cilju pružanja stručne pomoći u identifikovanju laboratorijskih kapaciteta i sprovođenju potrebnih stručnih obuka, kako za lekare specijaliste medicinske mikrobiologije, tako i za laboratorijske tehničare. I pored brzog razvoja novih tehnologija i automatizacije u laboratorijskom radu, ipak je teoretski i praktično edukovan specijalista medicinske mikrobiologije stub na kojem počiva savremena, tačna, brza i kvalitetna dijagnostika infektivnih patogena, a naročito onih sa pandemijskim potencijalom.

Ključne reči: Kovid-19, pandemija, mikrobiološka dijagnostika, zarazne bolesti

Uvod

Kovid-19 pandemija uzdrmala je i zdravstvene sisteme razvijenih zemalja, kao što su Francuska (1) i Španija (2), ukazujući time na slabosti njihove koncepcije zdravstvene zaštite, i pored činjice da ove zemlje imaju daleko veće finansijske resurse. Zato je i očekivano da se i u našem zdravstvenom sistemu, gde su finansijski resursi mali i ograničeni, brzo prikažu ključni nedostaci, kao što je nedostatak savremene opreme za brzu i tačnu mikrobiološku dijagnostiku, edukovanog kadra i adekvatnog laboratorijskog prostora.

Decenijsko zanemarivanje sistema javnog zdravlja, gde se u postojećem Zakonu o javnom zdravlju (3), medicinska mikrobiologija (ranije mikrobiologija sa parazitologijom), kao specijalizacija, i ne pominje, pokazalo je da je u ovom trenutku ova specijalizacija važna, kao i specijalizacija iz epidemiologije, kada je u pitanju brz i adekvatan odgovor u dijagnostici infektivnih patogena (3).

Zaposleni zdravstveni radnici i saradnici, koji rade u sistemu javnog zdravlja, čine tek 2% ukupno zaposlenog zdravstvenog kadra u Republici Srbiji (RS) (izvor: Institut za javno zdravlje RS). Oni nisu ugovoreni radnici preko Republičkog fonda za zdravstveno osiguranje (RFZO) i Ministarstva zdravlje (MZ) RS, već se finansiraju sredstvima koja Instituti i Zavodi obezbeđuju ugovorima sa RFZO. To znači da lične dohotke za lekare, specijaliste medicinske mikrobiologije u Zavodima i Institutima za javno zdravlje, ne obezbeđuje RFZO, već se oni finansiraju najvećim delom iz mikrobioloških dijagnostičkih usluga (RFZO), programske aktivnosti iz domena javnog zdravlja (MZ RS) i uslugama za komercijalno tržište.

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WILL THE COVID-19 PANDEMIC CHANGE ANYTHING? – A VIEW FROM THE ANGLE OF EXPERTS IN MICROBIOLOGY

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SUMMARY

The COVID-19 pandemic has shaken the majority of health systems around the world, pointing out that it is necessary to invest in the modern equipment for molecular diagnostics, as well as to provide an adequate space within microbiological laboratories for this form of diagnostics, according to the standards that are required for such procedures. It is also necessary to increase the number of specialists within the field of medical microbiology, especially in those laboratories where the lack of experts is at an alarming level. Investing in the diagnostic branches of medicine (and microbiology, as well) provides the optimal health protection: fast and correct diagnosis, the application of the adequate antimicrobial therapy as soon as possible, resulting in greater chances for curing. Furthermore, this leads to fewer hospital days and fewer deathly outcomes, while for infirmary patients this means a faster diagnostic procedure and a decrease in the number of specialist examinations, which are sometimes unnecessary. During the Covid-19 pandemic, The Section of Microbiologists of the Serbian Medical Society as well as the Institute for Microbiology and Immunology of the Faculty of Medicine of Belgrade University, have made themselves available to the Ministry of Health of The Republic of Serbia for the identification of potential laboratory capacities and for conducting necessary experts training, for doctors specialists and for laboratory technicians, as well. Although new technologies develop fast and the laboratory work becomes automated, in theory and in practice as well, an educated expert in medical microbiology still presents the main pillar that stands up for a precise, modern, quick and high-quality diagnosis of infective pathogens, especially those with pandemic potential.

Key words: COVID-19, pandemic, microbiological diagnostics, infectious diseases

The Covid-19 pandemic has shaken even the health systems of developed countries, such as Spain (1) and Italy (2), pointing out the weak points of their concept of health protection, although these countries have a lot greater financial resources. Therefore, it does not come as a surprise that our health system, in which the resources are scarce and limited, had to tackle problems, such as the lack of modern equipment for fast and precise microbiological diagnostics, educated personnel and an adequate laboratory space.

The system of public health has been neglected for decades. In the existing Law on Public Health (3), medical microbiology (previously microbiology with parasitology) is not even mentioned as specialization. However, it turned out that this specialization is very important, as well as the specialization in epidemiology, for a quick and adequate response in relation to diagnostics of infectious pathogens (3).

Employed health care workers and associates, who work in the public health system, make only 2% of the total health care personnel in The Republic of Serbia, according to The Public Health Institute of The Republic of Serbia. The National Health Insurance Fund does not provide salaries for doctors, specialists of medical microbiology at Public Health Institutes. However, they are financed, for the most part, from the microbiological diagnostic services (The National Health Insurance Fund), the program activities from the public health domain (Ministry of Health, RS), or from the services for the commercial market.

U takvoj konstelaciji, na samim Institutima i Zavodima za javno zdravlje je da obezbede savremenu opremu za dijagnostiku, održavaju i osavremenjuju laboratoriju, finansiraju troškove akreditacije laboratorija, kao i da obezbede kadar za obavljanje dijagnostičkih aktivnosti. Prema podacima Mikrobiološke sekcije Srpskog lekarskog društva (SLD), specijalizacija iz medicinske mikrobiologije je na listi deficitarnih specijalizacija (pored patologije, anesteziologije, radiologije, i dr.), a takođe, prosek starosti trenutno aktivnih zaposlenih specijalista mikrobiologa je veoma visok, što je obeshrabrujući podatak.

Zabrana zapošljavanja u prethodnom periodu, dodatno je otežala ionako tešku situaciju u pogledu odgovarajućeg broja specijalista mikrobiologije, gde imamo slučajeve da u pojedinim zavodima / institutima / bolničkim laboratorijama radi samo jedan specijalista medicinske mikrobiologije. Situacija je još ozbiljnija u mikrobiološkim laboratorijama koje funkcionišu u nekim opštim bolnicama (sekundarnog nivoa zdravstvene zaštite), kao službe pri sektoru laboratorijske dijagnostike. U tercijarnim ustanovama (klinike/instituti), situacija je dosta bolja, jer se na tim nivoima zdavstvene zaštite, prepoznaje značaj brze, tačne i savremene dijagnostike infektivnih bolesti. Opremljenost naših mikrobioloških laboratorija, kako pri zavodima/institutima, tako i pri opštim bolnicama/klinikama je raznolika i zavisi od mnogo faktora: zainteresovanosti menadžmenta, motivisanosti i sposobnosti rukovodilaca laboratorija, učešća u projektima, kao i od finansijskog stanja ustanove.

Treba istaći kao pozitivan primer, projekat MZ RS i Evropske unije i Evropske agencije za rekonstrukciju pod nazivom „Unapređenje laboratorisjkih usluga u Srbiji“, koje je sprovela konsultantska kuća „Eptisa“ (2005-2008), kada su određene referentne laboratorije (RL) za kontrolu zaraznih bolesti, a laboratorije koje su učestvovale u pomenutom projektu dobile su pomoć u laboratorijskoj opremi, edukaciji kadra (26 obuka), kao i posebnoj edukaciji osoblja iz RL u raznim evropskim laboratorijama, za specifične poslove referentnih aktivnosti iz svog domena.

Tokom 2014. godine je MZ RS sprovelo projekat „Pruženje unapređenih usluga na lokalnom nivou“ (DILS projekat), u okviru koga je izvršeno unapređenje rada Nacionalnih RL kroz povećenje njihove vidljivosti i povezanosti. U toku 2018. godine realizovan je zajednički „Projekat bratimljenja“ (MZ RS, Institut za javno zdravlje Srbije „Dr Milan Jovanović Batut“ i Nacionalni Institut za javno zdravlje Italije) pod nazivom „Poboljšanje kvaliteta sistema mikrobiološke dijagnostike u funkciji nadzora zaraznih bolesti u RS”. Izvršena je procena kapaciteta mikrobioloških laboratorija, identifikovani su nedostaci u odnosu na standarde u otkrivanju i potvrdi zaraznih bolesti i izrađene su preporuke za unapređivanje kvaliteta rada (4).

Samim završetkom specijalizacije i polaganjem specijalističkog ispita, ne završava se edukacija jednog medicinskog mikrobiologa. Tek kasnije, posle nekoliko godina rada, kada se sustignu znanje i iskustvo, neophodan je studijski boravak u savremenim, dobro opremljenim laboratorijama u inostranstvu, bar jedne (poželjno više) osoba iz laboratorije, jer će to doneti neprocenjiva iskustva, a kasnije koristi kroz bolju organizaciju i osavremenjivanje postojećih dijagnostičkih procedura. Takođe, neophodno je stalno unapređenje veština pohađanjem specijalizovanih praktičnih kurseva, kao oblika kontinuirane medicinske edukacije (KME), koje organizuje Institut za mikrobiologiju i imunologiju, Medicinskog fakulteta Univerziteta u Beogradu (npr. molekularna dijagnostika: RT-PCR engl. R*eal Time Polimerase Chain Reaction*, praktične radionice iz medicinske mikologije, parazitologije, bakteriologije i virusologije), što se u ovoj pandemijskoj situaciji pokazalo kao veoma važno. Laboratorije koje su od ranije imale uvedenu molekularnu dijagnostiku i posedovale aparate, brže su se uključile u proces detekcije SARS-CoV-2 virusa, RT-PCR metodom. U sklopu projekata Pokrajinskog sekretarijata za zdravstvo Vojvodine, svi Zavodi za javno zdravlje u Vojvodini dobili su RT-PCR aparate u toku 2013. godine (5).

Sticanje znanja iz medicinske mikrobiologije i boravak u savremenim mikrobiološkim laboratorijama je neophodno uvrstiti u plan i program određenih specijalizacija (npr: epidemiologija, infektivne bolesti, anesteziologija i dr.). Takođe, neophodno je dobro

In such a constellation, Public Health Institutes should provide modern equipment for diagnostics on their own, as well as maintain and modernize their laboratories, finance the expenses of laboratory accreditation, and provide personnel who can perform diagnostic activities. According to the data from the Microbiological Section of the Serbian Medical Society, the specialization in medical microbiology is on the list of deficient specializations (beside pathology, anesthesiology, radiology, etc.). Also, the mean age of the currently employed specialists of microbiology is very high, which is a discouraging fact.

The prohibition of employing the new cadre has made a difficult situation even more difficult regarding the appropriate number of specialists of microbiology. There are examples that only one specialist of medical microbiology works in certain institutes, and hospital laboratories. The situation is even more serious in microbiological laboratories in some general hospitals (secondary level of health care), which function within the department of laboratory diagnostics. In tertiary institutions (clinics, institutes), the situation is a lot better, because at this level of health care, the significance of quick, precise and modern diagnostics of infectious diseases is recognized. The state of equipment in our microbiological laboratories and at institutes, general hospitals, clinics is different and it depends on a lot of factors: managers' interest, motivation and skills of managers of laboratories, participation in projects, and the financial state of the institution.

One project should be emphasized as a positive example. It is the project of The Ministry of Health and the European Union, that is, the European Agency for Reconstruction. The project "Strengthening the Services of Public Health Laboratories in Serbia" was conducted by the consulting agency "Eptisa" (2005-2008), when reference laboratories were nominated for the control of infectious diseases, and laboratories-participants in the above-mentioned project were given help related to laboratory equipment, cadre education (26 trainings), as well as special education of cadre from reference laboratories in different European laboratories regarding the specific activities from their field of work.

In 2014, The Ministry of Health of the Republic of Serbia conducted the project "Delivery of Improved Local Services" (DILS project), within which the National reference laboratories improved their work by increasing their visibility and connectivity. In 2018, the mutual project "Twinning light project" (The Ministry of Health, Public Health Institute of Serbia "Dr Milan Jovanovic Batut" and the National Public Health Institute of Italy) under the title "Improving microbiology diagnostic system quality in the function of surveillance of communicable diseases in the Republic of Serbia". The capacity of microbiological laboratories was estimated, weaknesses were identified regarding standards of identifying and confirming infectious diseases and recommendations for improving the quality of work were made (4).

The education of medical microbiologists is not completed when they finish specialization and pass the specialist exam. Later, after a few years of work, when they have knowledge and experience, a study visit is necessary in modern, well-equipped laboratories abroad, of at least one person (desirably more people) from the laboratory, because it will bear priceless experience, and later benefits through better organization and modernization of existing diagnostic procedures. Also, skills should be improved by attending specialized practical courses, as a form of continuous medical education, which is organized by the Institute of Microbiology and Immunology of the Faculty of Medicine, University of Belgrade (for example, molecular diagnostics: RT-PCR – Real-Time Polymerase Chain Reaction), practical workshops from medical mycology, parasitology, bacteriology, virology), which has proved to be very important in this situation of the pandemic. Laboratories, which had introduced molecular diagnostics before and which had equipment, joined faster the process of detecting the SARS-CoV-2 virus with RT-PCR method. All Pubic Health Institutes in Vojvodina got RT-PCR machines in 2013, within projects of the Provincial Secretariat for Health Care of Vojvodina (5).

Acquiring knowledge in the field of medical microbiology and staying in contemporary microbiological laboratories should necessarily

poznavanje i razumevanje dijagnostičkih tehnologija u oblasti mikrobiologije, jer će to umnogome doprineti boljem stručnom razumevanju i saradnji ovih, po prirodi posla, blisko povezanih specijalizacija, a i brži razvoj mikrobioloških dijagnostičkih metoda i tehnologija to diktira. Radna mesta zdravstvenih radnika (specijalista/subspecijalista iz oblasti medicinske mikrobiologije, strukovnih medicinsko laboratorijskih tehnologa, laboratorijskih tehničara), kao i zdravstvenih saradnika (molekularnih biologa), koji neposredno učestvuju u izvođenju dijagnostičkih metoda, nisu bili uniformno klasifikovana kao radna mesta pod rizikom (najpre biološkim, a onda i hemijskim), što se podrazumeva, već je u svakoj zdravstvenoj ustanovi, prema internom Aktu o proceni rizika bilo različito. U realnosti smo svedoci da radna mesta dva medicinska mikrobiologa, koji rade identične poslove, budu različito klasifikovana; jedno radno mesto je pod rizikom, drugo ne. Prvo, metodologija procene nije rađena po „Specijalizovanoj metodologiji za procenu rizika u zdravstvenim ustanovama“ predloženoj od strane MZ RS i Instituta za medicinu rada RS „Dr Dragomir Karajović“(6), a nisu ispunjeni ni potrebni uslovi, da ustanova koja vrši procenu mora da poseduje licencu za obavljanje poslova ispitivanja uslova radne okoline - hemijskih i fizičkih štetnosti (osim jonizujućeg zračenja, mikroklime i osvetljenosti). Ipak, ova pandemija je donela pozitivne promene, te je ovaj problem nedavno rešen izmenama u sklopu najnovijeg akta MZ RS (7).

Neophodno je uložiti sredstva u savremenu opremu za molekularnu dijagnostiku, kao i obezbediti adekvatan prostor u mikrobiološkim laboratorijama za ovaj vid molekularne dijagnostike, po normativima koji su za to predviđeni. Takođe, potrebno je povećati broj specijalista medicinske mikrobiologije, naročito u laboratorijama gde je nedostatak kadra alarmantan. Dobro je poznato da je ulaganje u dijagnostičke grane medicine (gde spada i mikrobiologija), ključni faktor za optimalnu zdravstvenu zaštitu: brzo postavljanje tačne dijagnoze, primenu adekvatne antimikrobne terapije u kratkom vremenskom periodu, a samim tim postoje veće šanse za izlečenje, smanjenje broja bolničkih dana i broja umrlih, a za ambulantne pacijente obezbeđuje se brže postavljanje dijagnoze i manji broj raznih, nekada nepotrebnih specijalističkih pregleda. U toku pandemije Kovid-19, Sekcija mikrobiologa SLD i Institut za mikrobiologiju i imunologiju Medicinskog fakulteta Univerziteta u Beogradu, stavili su sav svoj kadar na raspolaganje MZ RS, dali su stručnu pomoć u identifikaciji laboratorisjkih kapaciteta i sproveli potrebnu stručnu obuku, kako za lekare specijaliste medicinske mikrobiologije, tako i za laboratorijske tehničare.

Na kraju, najvažniji i najbitniji resurs predstavlja osoblje - kompetentan kadar. I pored brzog razvoja novih tehnologija, automatizacije u laboratorijskom radu (koju je i Kovid-19 pandemija dodatno uvela u našu struku, zbog potreba molekularne dijagnostike), ipak je teoretski i praktično edukovan specijalista medicinske mikrobiologije stub na kojem počiva savremena, tačna, brza i kvalitetna dijagnostika infektivnih patogena, a naročito onih sa pandemijskim potencijalom. Zato je opravdano bilo pitanje postavljeno na početku ove pandemije „Da li smo mi to zapostavili laboratorije, a one su ključna karika u lancu borbe protiv virusa?“. Kliničke mikrobiološke laboratorije su vitalna karika u lancu aktivnosti potrebnih da se populacija odbrani od pretećih agenasa, pa i novog koronavirusa (8). Svakako, potrebno je razvijati i druge ciljane strategije za unapređenje pripremljenosti zajednice i države, kao što su promovisanje promena ponašanja i poboljšanje odlučivanja o upravljanju rizicima (8).

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be included into the plan and program of certain specializations (e.g. epidemiology, infectious diseases, anesthesiology, etc.). Also, knowledge and understanding of diagnostic technologies in the field of microbiology are necessary, because this will contribute to better professional understanding and cooperation of these, closely connected specializations. Faster development of microbiological diagnostic methods and technologies dictates it, as well. Positions of health care workers (specialists/subspecialists in the field of medical microbiology, professional medical laboratory technologists, laboratory technicians) and health care associates (molecular biologists), who directly take part in running diagnostic methods, have not been uniformly classified as positions at risk (first of all, biological, and then chemical), but differently in each health care institution, according to the Act on Risk Assessment. In reality, we witness the fact that job positions of two medical microbiologists, who do the same work, are differently classified; one position is at risk, and the other is not. Firstly, the methodology of assessment was not done according to the "Specialized methodology for risk assessment in health care institutions", proposed by the Ministry of Health of The Republic of Serbia and the Serbian Institute of Occupational Health "Dr Dragomir Karajovic" (6), while the necessary conditions were not fulfilled, meaning that the institution, which does the assessment, has to possess the license for examining the conditions of the work environment –chemical and physical harm (beside ionizing radiation, microclimate and lighting). However, this pandemic has brought some positive changes, and this problem has been solved recently within the latest Act of the Ministry of Health of the Republic of Serbia (7).

Resources should be necessarily invested in the modern equipment for molecular diagnostics, and an adequate space should be provided in microbiological laboratories for this kind of molecular diagnostics, according to the set standards. Also, the number of specialists of medical microbiology should be increased, especially in those laboratories where the lack of experts is at an alarming level. It is well-known that investing in diagnostic branches of medicine (and microbiology, as well) is a key factor for optimal health protection: setting the precise diagnosis quickly, administration of adequate antimicrobial therapy as soon as possible, and therefore, chances for curing are bigger, the number of hospital days and deathly outcomes decreases, and for infirmary patients faster diagnosis is secured and a smaller number of sometimes unnecessary examinations, as well. During the Covid-19 pandemic, The Section of Microbiologists of the Serbian Medical Society and the Institute for Microbiology and Immunology of the Medical Faculty of the University of Belgrade, have made their personnel available to the Ministry of Health of The Republic of Serbia; have helped professionally in identifying the laboratory capacities and conducted the education trainings for doctors specialists of medical microbiology, and for laboratory technicians, as well.

In conclusion, the most important resource is the personnel – the competent cadre. Although, the fast development of new technologies and the automation of laboratory work (which has been brought into our field by the Covid-19 pandemic, due to the needs of molecular diagnostics) are important, in theory and practice, an educated specialist of medical microbiology presents the main pillar that stands up for a modern, precise, quick, and high-quality diagnosis of infective pathogens, especially those with the pandemic potential. Therefore, the question, which was posed at the beginning of this pandemic, was justified: "Have we neglected laboratories, which present the key ring in the chain of struggle against this virus?" Clinical microbiological laboratories are a vital ring in the chain of activities necessary to defend the population from the threatening agents, and the novel corona virus, as well (8). Certainly, it is necessary to develop other targeted strategies for the promotion of society and state preparedness, such as promoting the changes of behavior and improving decision making about risk management (8).

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Parts of the manuscript are: title page, summary in Serbian and English with keywords in Serbian and English, text of the manuscript (Introduction, Methods, Results, Discussion, Conclusion, Literature, Acknowledgment) and appendices.

The manuscript should be written briefly and clearly and abbreviations used only for very long names and for names known as abbreviations (eg AIDS, HIV, etc.).

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Give the name of the manuscript (in capital letters), full names of the authors, their affiliation. Associate author names with institution names indexed by Arabic numerals. Also provide the first and last name for the corresponding author, their institution, institution address, telephone number and e-mail address.

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and in review papers, the summary is descriptive (without subsections). The third page is identical to the other, but is in English.

Introduction/Aim
The introduction should be clear and directly related to the subject of the research. It should provide the most important information about the problem that is being dealt with, as well as what has been investigated so far about the problem, what is known and what is unknown, or little known, or if there is controversial information. After the introductory notes, the aim of the paper should be stated.

Methods
In this section, the authors describe how the study was conducted, explain the choice of methods and design of the research. The sub-sections of the methods may be: study design (eg quantitative or qualitative research, descriptive or analytical or experimental study, etc.), choice of respondents (inclusion and exclusion criteria from the study), ethical aspects (the number under which the study was approved by the ethics committee), research instruments (method of data collection, specificity of instruments used), and statistical analysis of the data (types of tests). It is important to provide literature data for known methods, including statistical methods.

The results
Describe the results of the research presented in a logical order through tables, charts and illustrations (appendices are cited after the Literature).

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Compare the results of your research with the results of other relevant research already published (if possible not older than five years).

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Acknowledgment
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Appendices
Appendices include tables, pictures (photos, drawings, diagrams, charts) and video attachments. All appendices must be in Serbian and English. There must be a title above all appendices for each appendix. All appendices are indicated by Arabic numerals in the order in which they appear in the text. The use of abbreviations in the headings or any part of the appendix must be explained below.

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