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COVID-19 Microbiological and Radiological Diagnostics

Mikrobiološka i radiološka dijagnostika COVID-19

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Summary

The purpose of this paper is to present SARS-CoV-2 diagnostic methods and their optimal implementation. The main routine microbiological diagnostic tools are molecular and serological methods. Sensitivity and utilisation of molecular methods is the best at the disease onset, while serological methods are the best if used at least 5-7 days after disease onset. Positive molecular test result (real-time polymerase chain reaction; RT-PCR) must be interpreted in the clinical presentation context. A sampling site for molecular analysis is based on disease severity; upper and lower respiratory tract samples are used for mild and severe presentations, respectively. Virus cultivation is the only method of proving the existence of a viable virus in a tissue sample, but due to the method complexity, it is not a part of a routine process. Ground glass opacities with or without reticular pattern and/or consolidation are typical findings for COVID-19 pneumonia. Multi-slice computerized tomography (MSCT) is a superior radiological method for performing X-ray of the chests.

Sažetak

Ovaj članak ima za cilj prikazati dijagnostičke metode SARS-CoV-2 infekcije, kao i njihovu optimalnu primjenu. Glavne rutinske mikrobiološke dijagnostičke postupke predstavljaju molekularne i serološke metode. Osjetljivost i primjena molekularnih metoda najbolja je na početku bolesti, dok je primjena seroloških metoda najprikladnija barem 5-7 dana nakon početka bolesti. Pozitivan rezultat molekularnog testa (lančana reakcija polimeraze u stvarnom vremenu, engl. real-time polymerase chain reaction; RT-PCR) mora biti protumačen u skladu s kliničkom prezentacijom. Mjesto uzorkovanja za molekularnu analizu ovisi o težini bolesti; uzorci iz gornjeg i donjeg respiratornog trakta koriste se pri blagim i teškim prezentacijama, navedenim redoslijedom. Uzgoj virusa na staničnoj kulturi jedina je metoda koja se primjenjuje u svrhu dokazivanja živog virusa u uzorku tkiva, međutim, metoda se ne izvodi rutinski zbog složenosti metodologije. Promjene po tipu mliječnog stakla s ili bez retikularnog uzorka i/ili konsolidacija tipične su pojave kod COVID-19 upale pluća. Višeslojna kompjutorizirana tomografija (engl. *multi-slice computerized tomography;* MSCT) predstavlja superiornu metodu, u odnosu na klasični RTG srca i pluća.

Introduction

In December 2019, a novel Coronavirus was identified as the cause of a cluster of pneumonia cases in Wuhan, China. Over the following months, it has rapidly been spreading across the globe, causing the biggest pandemic of the 21st century. The virus was named SARS-CoV-2 (Severe Acute Repiratory Syndrome Coronavirus-2), while the disease was named COVID-19 (Coronavirus Disease 2019). At the moment of writing this article, it has infected over 24.000.000 individuals and caused more than 800.000 fatalities worldwide, of which nearly 2.000.000 cases and 180.000 deaths belong to the European Union/European Economic Area and the United Kingdom (UK)[1]. The medical and scientific community are witnessing a worldwide collaborative effort in understanding the virus and the disease, with hundreds of new scientific publications being available daily, thus expanding on knowledge concerning the spread of the virus and its effects on individuals and society. Little is known concerning the period of infectiousness, persistence and efficiency of long-term immunity, while the targeted treatment and vaccines are not yet available and are thus a subject of intensive research. The focus of this

article is a narrative review of COVID-19 diagnostic methods with special attention devoted to relationship and clinical implications of specimen type, the timing of sampling, viral load, duration of viral shedding, disease severity, patient characteristics and kinetics of sample positivity.

Epidemiology and Clinical Presentation

Disease severity ranges from asymptomatic and mild to critical and fatal. According to data from the Chinese CDC, gathered from 44.500 confirmed infections, 81% of cases are mild (no or mild pneumonia), 14% severe (dyspnea, hypoxia, more than 50% of lung involvement on imaging with 24-48 hours) and 5% critical (respiratory failure, shock, dysfunction of multiple organs). Overall case fatality rate is 2.3%; 14.8% in patients aged 80 or more, 8.0% in patients aged 70-79 and 49.0% in critical cases[2]. ECDC reports that 28% of all COVID-19 cases in the European Economic Area and the UK were hospitalized (median age 57), while 14% of hospitalized patients required intensive care and/or respiratory support (median age 64). It is estimated that 24% of hospitalized patients died, and almost all of them were aged 60 or more[1].

Diagnosis

According to the WHO guidelines, any patient with a new onset of fever and/or respiratory symptoms should be considered as a patient suffering from acute SARS-CoV-2 infection and specific microbiological diagnostics is needed for confirmation. Infection is more probable, if there is no alternate diagnosis explaining clinical features in details, if the patient travelled to an area with a high rate of local SARS-CoV-2 transmission or if the patient had close contact with confirmed or suspected case of COVID-19 in the last 14 days[3].

Currently, the diagnosis of SARS-CoV-2 infection can be established by using molecular and serological tests. Although not used routinely, virus isolation in cell culture is another valuable diagnostic method. Molecular diagnostic method is the most widely used method due to high sensitivity during pre-symptomatic and the first few symptomatic days, speed and procedure simplicity. However, since molecular methods can only verify the presence of viral nucleic acid, a positive test does not necessarily mean the presence of a viable virus. Therefore, the test results are to be interpreted in the clinical presentation context. ECDC guidelines recommend molecular testing of the upper respiratory tract specimens for diagnosing acute infection in individuals with symptoms consistent with COVID-19[4]. The lower respiratory tract specimens

are used in more severe clinical presentations. The serological methods are supplementary since they can generally be used 5-7 days after the symptoms onset, due to the dynamics of immune response and antibodies production. The serological methods can be used when molecular test results are inconclusive, in addition to being a useful indicator of the previous infection. ECDC does not recommend making the diagnosis of acute infection solely by applying serological methods[4]. Virus isolation in a cell culture is a definite proof of a viable virus presence, but due to a complex and long-lasting procedure, it is not appropriate for a routine use on a large number of samples. Another less utilized method of COVID-19 diagnostics is antigen detection. It uses the same specimens as molecular methods, but its advantages are rapid availability of results and point-of-care diagnosis. Even though it has a specificity of more than 99%, it is not used routinely due to the sensitivity of only 55% in comparison with molecular methods[5].

Molecular Diagnostics

The basis of diagnosing acute SARS-CoV-2 infection is the detection of viral RNA in clinical samples by conducting nucleic acid amplification tests (NAAT), most commonly reverse transcription polymerase chain reaction (RT-PCR). Several in-house protocols have been developed. The protocols, most commonly used in Europe, test the presence of the Envelope *(E)* gene used for SARS-CoV-2 screening and RNA-dependent RNA polymerase *(RdRp)* gene used for confirmation. The other most common target genes in various protocols include the Nucleocapsid *(N)* and Open reading frame 1 *(Orf1)* gene[6]. At the pandemic onset, the WHO and other agencies recommended at least two independent targets to be positive for declaring a person infected with SARS-CoV-2 due to public health implications. With confirmed autochthonous cases within a country, a single positive target is considered enough. NAATs are considered highly specific and sensitive, even though various protocols differ in their sensitivity up to a 1000-fold[7]. Additional factors influencing sensitivity include a type of clinical specimen, the timing of specimen sampling, viral load, patient's characteristics, disease severity, sampling technique, sample transportation, storage and analysis methods. The most common samples for RT-PCR confirmation of viral presence are upper and lower respiratory tract swabs and specimens, although viral RNA has also been detected in blood, urine and faeces. Cycle threshold (Ct) is the number of reaction cycles in real-time PCR, required to obtain a fluorescent signal and it is inversely correlated with viral load in samples;

the lower Ct value number, the greater the quantity of viral RNA. Generally, the values below the threshold of Ct=38 are considered positive but depend upon implementation and validation of each assay in each laboratory. Due to this and because the sample quantity varies with each individual swab of the respiratory tract, Ct is a highly variable and incomparable value.

Sampling Time Point and Sample Choice

It is considered that detectable viral shedding begins 1-2 days prior to the symptoms onset, however, Arons et al.[8] detected and isolated the virus in upper respiratory tract specimens 6 days prior to the symptoms onset. It should be noted that the participants were nursing home patients, and possible reasons for a long duration of RNA detection prior to the symptoms onset could be a small infectious dose, an alternate mode of virus entrance into organism or immunosenescence. Further validation requires studies observing suspected individuals in the general population.
Since COVID-19 primarily affects the respiratory system, the most common samples for routine testing of suspected individuals are oropharyngeal and nasopharyngeal swabs. Several studies demonstrated higher sensitivity of nasopharyngeal swabs, while studies that sampled both sites simultaneously revealed low detection consistency between the two sites, but higher overall detection sensitivity[9, 10].

According to data obtained by several studies[9-12], Table 1 represents rates of PCR positive respiratory specimens of mild and severe inpatients during the first 4 weeks of disease. The positive samples rates of both mild and severe cases are highest in the first week and have similar values, but in the following weeks, severe cases positive rates show a more gradual decline than mild cases rates. Oropharyngeal swab rates show a more rapid decline, while nasopharyngeal swab rates exhibit a more gradual decline[9, 10, 12]. The combined positive rate of nasopharyngeal and oropharyngeal swabs is overall higher than rates of individual sites, most likely due to a previously described low level of consistency between the two sites. The combined positive rate of saliva (upper respiratory tract specimen) and sputum (lower respiratory tract specimen) specimens is higher than individual rates of upper and lower respiratory tract specimens, also most likely due to a low level of consistency between the sites. The rates of positive samples from the lower respiratory tract (sputum, bronchoalveolar lavage fluid - BALF) during the first 4 weeks are higher than the rates of samples from the upper respiratory tract. Another sample for diagnosing COVID-19 is a self-collected lower nasal swab, and its advantages are reduced risk of health care personnel virus exposure and reduced usage of personal

protective equipment. The sensitivity of the method is 100%, specificity 95%, but the study included only 30 patients, while the method of sample collection was not described in detail[13].

Based on this data, several observations can be made. The probability of RNA detection in an upper respiratory tract sample appears to be the highest if patients present it during the first week of symptomatic disease, it declines rapidly for mild cases and gradually for severe cases in the following weeks. Nasopharyngeal swab has a high detection rate during the first week of the disease, and although oropharyngeal swab has somewhat lower detection sensitivity, both sites are easy to sample, and the detection sensitivity increases when combining both site samples in the same patient. Although sputum proved to be the specimen with the highest detection sensitivity along with a steady decline rate throughout the disease course, currently it is not a recommended sample for routine use because the majority of patients do not produce sputum and cough induction for sputum production is not advisable. Nevertheless, the lower respiratory tract samples are used for diagnosing the severe cases and the result can be positive, even in later disease stages. It is worth noting that the patients with high clinical and radiological suspicion and negative PCR test should be isolated and retested, while the serological investigations should also be considered in these patients. Repeated testing (up to three times) increases the positivity rate up to 12%, from 85.9% to 97.5%, while retesting beyond the third time does not yield a significant rate increase. The patients with initially positive samples are more likely to have or progress to severe disease[14, 15]

Implications of Viral Load in Samples

Generally speaking, the rates of the positive respiratory tract samples can be correlated to viral loads, and the comparison can be performed between mild and severe presentation groups. Most studies show that viral loads of inpatients with mild disease are at their peak during the first or second week of the disease, after which they gradually decline, while some observed consistent load during the first three weeks of the disease[10, 11, 16-18]. The viral loads in the lower respiratory tract specimens of severe cases are initially higher than in mild cases and remain high throughout the disease course[10, 11, 16]. Some studies observed no difference of viral loads in the upper respiratory tract samples of mild and severe cases, but did observe significantly higher viral loads in older patients[16, 19]. Hu et al. analysed the rate of negative conversion in patients with Ct values below 30 and 30 or above during the first three weeks of the disease and found no difference between the two groups, but found that chest tightness

leads to longer conversion time[19]. To the best of our knowledge, this is the only study directly investigating the link between viral load and duration of RNA detection in samples. In clinical practice, measuring viral loads in lower respiratory tract samples (i.e. sputum) could be helpful to discriminate patients requiring close monitoring and early antiviral treatment. However, most of the studies do not report exact values of viral loads, or do so in incomparable units of measure (log, number of virus copies per millilitre of sample and Ct values).

Nucleic Acid Detection Duration

At the pandemic onset, in order to discharge a patient from hospital the symptoms must have been resolved and two consecutive swabs negative. However, several studies report the third swab to be positive in 7-30% of the patients[9, 12, 19]. There has been no clear connection between this phenomenon and other parameters such as viral load, duration of positive samples or disease severity. It is unclear whether these patients shed infectious live virus, virions encircled by antibodies, viral nucleic acid, whether the test detects infected epithelial cells or the test results are false positive. With a highly sensitive method like real-time PCR, this – most likely – represents the remnants of viral replication still being detected even though there is no active virus production. Such observations were made for this method in other viral diseases as well, but were not considered problematic, since no quarantine was required. These issues further question duration of hospital stay, isolation and the potential for virus spreading from an individual with the two negative and subsequently positive samples after disease resolution.

Conflicting data exist on the duration of RNA detection in mild and severe cases. While some studies report median detection duration in the upper respiratory tract samples of mild cases to be 13-15 days[19, 20], the others report it to be 20-24 days[9, 12]. The latter duration is similar to the reported median detection duration of 21-22 days in both upper and lower respiratory tract samples of the severe cases[11, 20]. Although Zheng et al. found that RNA detection lasts longer in severe cases, To et al. found no correlation[11, 16]. Some patients' samples remain PCR positive even for a total of 5 weeks[12, 17, 18]. Several studies suggest that older patients have a prolonged period of virus elimination, possibly due to the immunosenescence, even though different age is reported as the threshold, from 45 to more than 65 years of age[9, 11, 12, 19]. Viral detection is also prolonged in patients who received glucocorticoids and in patients with comorbidities[11, 12]. All data were collected only from inpatients, who represent the more severe spectrum of disease, therefore the duration of viral shedding could be overestimated, as outpatients with mild disease were not periodically sampled.

Alternative Samples for SARS-CoV-2 Detection

There is evidence of faecal shedding of SARS-CoV-2. Zheng et al.[11] detected the virus in 59% of stool samples, and found that no difference in shedding between mild and severe cases. However, faecal shedding intensified from the second week and the duration of virus detection in the stool is longer than the detection in respiratory samples (22 vs. 18 days) [Table 1]. In a study of 84 confirmed COVID-19 hospital workers with mild disease, 31% had diarrhoea, the mean time for throat swab to turn negative lasted longer than in the non-diarrhoea group (12.5 vs. 9.2 days) and they had a higher percentage of positive control stool samples, although the difference was not significant (45% vs. 20%)[21]. Although SARS-CoV-2 RNA can be detected in stool, there is no clear evidence of the faecal-oral route of transmission.

Wolfel and Kujawski detected no RNA in the blood of mildly and moderately ill patients in their studies of 9 and 12 patients, respectively[17, 18]. Other studies report rates of RNA detection in the blood to be 15-66% for mild patients and 30-87.5% for severe patients with low values of viral load[11, 16, 20]. To the best of our knowledge, no study reported successful virus isolation from the blood. Several other bodily fluids were tested for the presence of viral RNA but only anecdotal evidence exists for the presence of RNA in urine and tears[11, 16-20].

Virus Cultivation

Virus cultivation in a cell culture is the only method which can prove whether the virus in a sample is still viable. Although this information could warrant prolongation or discontinuation of patient isolation, the method is complex, long-lasting, and the performing laboratory must meet Biosafety Level 3 requirements. Due to these issues, it is not appropriate for routine use on a large number of samples and is primarily used for research purposes. Virus cultivation from the upper respiratory tract specimens was studied in relation to disease day by several research groups. Arons et al.[8] isolated the virus from the samples taken from 6 days before to 9 days after the symptoms onset, Kujawski et al.[17] from specimens taken before prior to day 9 and Wolfel et al.[18] from specimens taken before prior to day 8. The unpublished data suggest that the virus isolation was not successful after day 10 in mild and moderate disease presentation, while the patients with a severe presentation rarely shed infectious virus up to day 20[22]. Based on these findings, the necessity of using person-

Table 1. Rates of SARS-CoV-2 PCR positive samples in inpatients according to specimen site, disease severity and sampling time.

Tablica 1. Stope uzoraka pozitivnih na SARS-CoV-2 metodom polimeraze lančane reakcije (PCR), podijeljene prema mjestu i vremenu uzorkovanja te težini bolesti.

Disease severity / Sample	PCR positive sample rates (%)							
	Week 1		Week 2		Week 3		Week 4	
	Severe	Mild	Severe	Mild	Severe	Mild	Severe	Mild
Nasopharyngeal swab	73	72	72	53	50	54	/	/
Nasopharyngeal swab	60	61	50	29	37	11	/	/
(combined naso/oropharyngeal, mild)		98*- / 100∞		69*- / 89∞		36*- / 66∞		30*- / 32∞
Saliva	/	/	/	/	/	/	56+	44+
Sputum	89	82	83	74	61	43	47+	
(combined sputum+saliva)	97+	92+	86+		71+	79+		
Bronchoalveolar lavage fluid	/	/	100	0	78	/	/	/
Stool	44+	29+	47+	50+	41+	59+	31+	56+

Unmarked data by Yang et al. (10);

* - data by Xiao et al. (9) represents combined nasopharyngeal and oropharyngeal sample results of mild cases;

+ - data by Zheng et al. (11) represents combined sputum and saliva samples and stool specimens' results of mild and severe cases;

∞ - data by Xiao et al. (12) represents combined nasopharyngeal and oropharyngeal sample results of mild cases.

al protective equipment could be reconsidered in these patients. The apparent correlation between the absence of specific antibodies and successful viral isolation may suggest the appearance of IgG antibodies in the serum as a possible time point for discharge of patients[18].

Serological Diagnostics

Due to a variable sensitivity of molecular methods during the course of COVID-19, the serological methods have been investigated as a possible supplement. Generally speaking, the IgM and IgG antibodies are considered to be the markers of acute and past infection, respectively. The IgA antibodies, in addition to being the acute infection markers, are also the mucosal immunity and respiratory tract infection markers. The serology sensitivity depends on host immune response, the timing of serum sampling, analysis method and assay quality, all of which vary among the manufacturers. The specificity depends on the type of antigen used for antibodies detection. Surface spike protein (S), its subunit receptor-binding domain (RBD) and internal nucleoprotein (NP) are the most common protein antigens used in detection of antibodies against SARS-CoV-2. The studies found almost no N protein antibody cross-reactivity of SARS-CoV-2 and other 4 human corona viruses, but found strong cross reactivity with SARS-CoV antibodies, possibly due to 90.5% sequence homology. Nevertheless, RBD is specific to SARS-CoV-2 and should therefore be used for a higher specificity[16, 23].

There are several methods for antibodies detection in serum samples. The classic solid-phase enzyme-linked immunosorbent assays (ELISA) have relatively high sensitivity and specificity, chemiluminescence immunoassay methods (CLIA) have comparable characteristics and lateral flow immunochromatographic assays (ICA) are affordable and easy to use, thus suitable for point of care testing. It is necessary to possess knowledge of the method's sensitivity and specificity, as well as its positive and negative predictive values while interpreting results.

According to antibody kinetics data from several studies, IgM and IgA antibodies are the early-stage markers. They appear towards the end of the first disease week, reach peak values in the second week and start to decline somewhere between the third and the fourth week. The IgG antibodies start to appear faintly in the first week, continue to increase in quantity throughout the third week and reach the plateau by the end of it[24-28] [Table 2]. Padoan et al.[29] compared the IgA and IgM levels in PCR confirmed COVID-19 patients and found persistently higher levels of IgA during the whole observation period with a peak level at days 20–22. To et al.[16] found the rate of the IgG positive samples to be higher than the rate of the IgM positive samples (94-100% vs 88-94%) 14 days after symptoms onset and Long et al.[24] found 100% of samples are IgG positive on days 17-19, while only 94.1% of samples are IgM positive on days 20-22. In compar-

ison, Lou et al.[26] found the cumulative rate of total antibodies and IgM was 100% on days 16 and 21, while the rate of IgG was 97.1% on day 29 [Table 2].

Pan et al.[28] investigated antibody positivity both in COVID-19 confirmed and suspected cases and found the overall rate of combined IgM and IgG positive cases in both groups to be 68.6% (69/86) and 63.6% (14/22), respectively. By detecting total antibodies (IgM, IgG and IgA) in the first week, the diagnostics sensitivity ranges from 60 to 75%[24-27]. Guo et al.[23] point out that the detection rate of COVID-19 cases is only 51.9% by using a single PCR test, but significantly increases to 98.6% when an IgM ELISA assay is applied to PCR-negative patients.

When comparing the onset of seropositivity between anti-RBD and anti-NP, To et al.[16] found that more patients had earlier seropositivity for anti-RBD than anti-NP for both IgG and IgM, while Sun et al.[25] found no differences between the groups. Several studies compared seroconversion time and antibody titer in severe and non-severe patients and found no significant difference between these groups[24, 26]. Only Sun et al. found that S-IgG was higher in the non-intensive care unit (ICU) group, and N-IgG was higher in ICU group[25].

In conclusion, the serological methods are an important supplement in COVID-19 diagnosis. Their sensitivity is at its peak when used 5 days after the disease onset, both independently or in conjunction with molecular methods. Serology is useful in distinguishing whether the patient is infected when clinical suspicion is high and molecular test is negative, and in situations when two or more molecular tests yield opposing results. It is yet unknown whether the IgG antibodies provide long-term immunity to an infected person. The serological methods utilisation also includes the asymptomatic cases detection, seroprevalence studies, detection of patients with convalescent serum for a potential use as therapy and monitoring of immune response to trial vaccines.

Radiological Diagnostics

The imaging modalities used in SARS-CoV-2 infection diagnosis are: chest x-ray (CXR), multi-slice computerized tomography (MSCT) imaging and lungs ultrasound. These methods are supplementary and a decision upon using one of them is based on the clinical setting, individual patients' needs and a meth-

Table 2. Rates of IgM and IgG anti-SARS-CoV-2 in suspected and confirmed COVID-19 patients according to the week of disease and type of serological method used.

Tablica 2. Stope IgM i IgG protutijela na SARS-CoV-2 u pacijenata kod kojih se sumnja na COVID-19 infekciju ili je potvrđena, podijeljene prema tjednu bolesti i vrsti serološke metode.

Anti-SARS-CoV-2		Positive samples rate (%)			
		Week 1	Week 2	Week 3	Week 4
I	PCR confirmed cases				
	Total antibodies	60.0+-69.4	89.9+-94.4	100.0+	100.0+
	IgM	40.0+-58.3	75.1+-94.4	89.5-94.1+	92.3+
	IgG	55.5+-69.4	84.8+-94.4	94.7-100.0+	100.0+
II	PCR confirmed cases				
	Total antibodies	64.1-75.0*	94.7*-98.7	100.0*	100.0
	IgM	33.3-58.3*	84.2*-86.7	89.5*	96.7
	IgG	33.3-66.7*	76.0-94.7*	100.0*	93.3
III	PCR confirmed cases				
	IgM	11.1	78.6	74.2	/
	IgG	3.7	57.1	96.8	/
	Suspected cases				
	IgM	22.2	33.3	57.1	/
	IgG	44.4	66.7	71.4	/

I – CLIA (Chemiluminescence assay): unmarked data by Suhandynata et al. (27), + - data by Long et al. (24);

II – ELISA (Enzyme-linked immunoassay): unmarked data by Lou et al. (26), data for weeks 3 and 4 is represented together, *- data by Sun et al. (25);

III – ICG (Immunochromatographic assay): unmarked data by Pan et al. (28).

od's availability. Compared to MSCT, the chest X-ray is a less sensitive method of detecting COVID-19 pathology of the lungs. Nevertheless, it is usually the first imaging method performed on patients as it is a useful triage tool and has a role in patient's follow-up. Just like the other viral pneumonias, COVID-19 pneumonia causes lung opacities in more than one lobe and, therefore, a multi-focal air-space disease is a significant finding. The changes such as ground-glass opacities (GGO) visible on MSCT are very hard to detect in a CXR correlate, while the reticular opacities in the GGO regions are more easily detectable on a standard CXR[30]. A large study, performed at multiple university clinical centres in the greater New York City area on 636 patients in an outpatient care setting, showed that the chest X-ray findings may be normal in patients with mild and moderate disease. The vast majority of patients included in this study (566/636, 89%) had either normal or only mildly abnormal CXRs[31]. The consensus for imaging published by the Fleischner Society states that in patients with mild clinical features, imaging is indicated after a positive viral test if the patient has risk factors for disease progression. In patients with moderate to severe clinical features, the imaging is indicated after the introduction of a positive viral test if the patient is at risk of pulmonary status worsening[32].

The chest MSCT has an important role in detecting lesions caused by SARS-CoV-2, treatment evaluation and patient's follow-up. The studies on diagnostic MSCT from Italy and China report sensitivity around 97%, specificity from 25 to 56% and accuracy around 70%[33, 34]. The findings depend on the disease stage; from the first to the seventh day of the disease, the most common findings are ground-glass opacities [Figure 1], a combination of GGO and reticular pattern (the „crazy-paving pattern") [Figure 2] or GGO and consolidation with air bronchogram [Figure 3][35]. The lesions were predominantly distributed in posterior and peripheral parts of the lungs bilaterally.[36] From the 8th to the 14th day, the lesions become denser and multilobular, while the progression and absorption signs are simultaneously present. After approximately fourteen days, the abnormalities start to decrease and repairing signs, such as bronchal distortion, subpleural line and fibrotic strips are present[37]. There seems to be no positive correlation between the CT findings and disease severity[33]. Some parameters, such as blood lymphocyte count and SpO_2 have shown a statistically significant negative correlation, meaning: the higher the CT stage, the lower the lymphocyte count and SpO_2[36].

In a retrospective study by Ai et al. on a sample of 1014 patients, the chest CT images of 308 patients (mean

Figure 1. Thoracic multi-slice computerized tomography (MSCT) shows „ground glass" opacities (red square) in the upper left lobe in a patient with COVID-19 pneumonia on the fifth day after the symptoms onset (temperature 38.6 °C and malaise).

Slika 1. Višeslojna kompjutorizirana tomografija torakalne regije prikazuje zasjenjenja po tipu „mliječnog stakla" (crveni kvadrat) u gornjem lijevom režnju kod pacijenta s COVID-19 upalom pluća u petom danu nakon pojave simptoma (povišena tjelesna temperatura do 38.6°C i slabost).
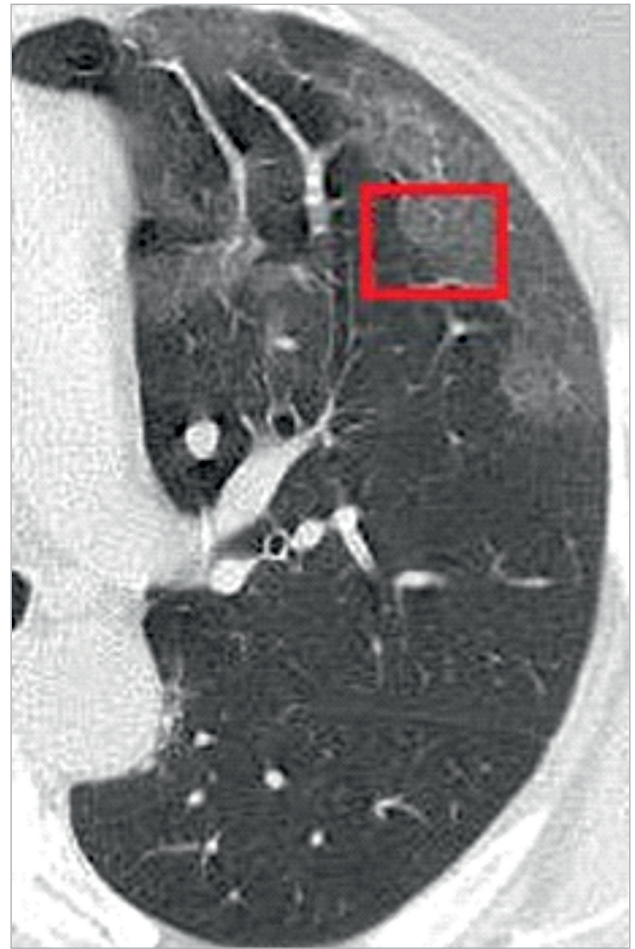
age 47 ± 14 years, 48% male population) suggested COVID-19, while their RT-PCR assays from throat swab samples were negative. Most patients (83%) had bilateral lung lesions consisting of ground-glass opacities and consolidations[34].

A possible reservoir of SARS-CoV-2 are asymptomatic or oligosymptomatic patients. Several studies showed that asymptomatic cases with a history of exposure lead to MSCT lesions confirmed cases. The lung opacities on MSCT were found in 44 (54%) out of 82 asymptomatic patients from the Cruise Ship „Diamond Princess", one of the largest clusters of COVID-19 patients outside China[38]. A study on asymptomatic patients from Wuhan showed that predominant

Figure 2. Thoracic multi-slice computerized tomography (MSCT) reveals diffuse, bilateral „crazy paving” pattern (red square) in a patient with COVID-19 pneumonia that started eight days earlier with a dry cough, temperature up to 39 °C and headache.

Slika 2. Višeslojna kompjutorizirana tomografija torakalne regije prikazuje difuzni, obostrani uzorak „ludog poploćenja” (crveni kvadrat) kod pacijenta s COVID-19 pneumonijom koja je započela prije 8 dana suhim kašljem, povišenom tjelesnom temperaturom do 39°C i glavoboljom.
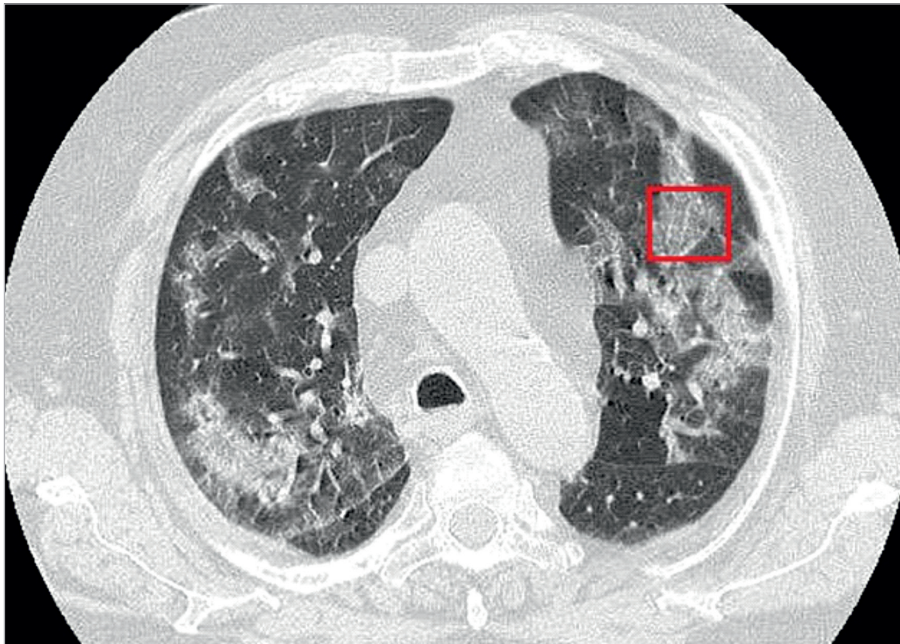

Figure 3. Thoracic multi-slice computerized tomography (MSCT) performed on the tenth day after the symptoms onset (temperature 38.2 °C, chest pain and shortness of breath) depicts bilateral, peripheral consolidations in lower lobes (red square).

Slika 3. Višeslojna kompjutorizirana tomografija torakalne regije učinjena osmi dan nakon pojave simptoma (povišena tjelesna temperatura do 38.2°C, bol u prsima i kratkoća daha) prikazuje obostrane, periferne konsolidacije u donjim režnjevima (crveni kvadrat).
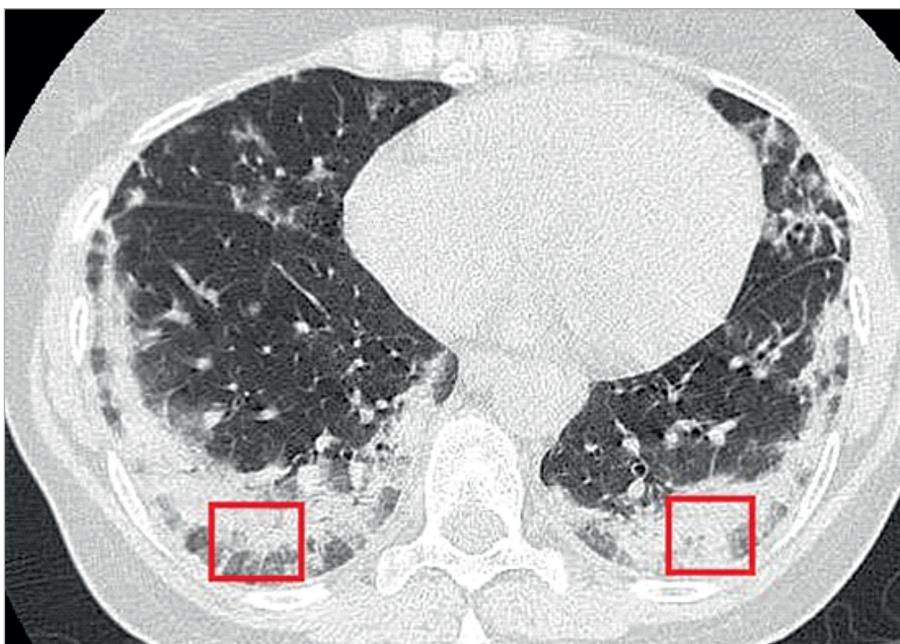
features of these cases were GGO with peripheral distribution, unilateral, mostly involving one or two lobes, combined with a subpleural curvilinear line, fine reticulation, air bronchogram, halo sign or vascular enlargement signs[39].

Although RT-PCR is a fairly sensitive method of detecting SARS-CoV-2, there is a small percentage of false negative samples. This can lead to poor containment of infected individuals and uncontrolled disease spreading, due to the highly contagious virus nature. MSCT has a significant role in the diagnosis of COVID-19 in these cases, particularly in the presence of symptoms and epidemiological exposure, ultimately allowing for timely isolation and proper treatment. The MSCT limitations are high radiation dosage compared to CXR and the need for disinfection of MSCT machine after use, which may lead to delays in diagnostics and work overload in case of MSCT being the primary screening tool.

The lungs ultrasound can be helpful in the evaluation of patients with suspected COVID-19 infection. The advantages over MSCT and CXR are the convenience of point of care diagnosis, no radiation and less utilization of personnel, such as radiologic technicians and transport staff. Also, it seems that the lungs ultrasound can detect lesions earlier if the lesions are adjacent to the pleura. The typical findings of COVID-19 pneumonia are glass rockets with or without the Birolleau variant, confluent B lines, thick irregular pleural lines, and subpleural consolidations[40].

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Conflict of interest

The authors have no conflicts of interest to declare.

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