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Immunology and Microbiology to the Article “SARS-CoV-2 Infection”

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Abstract

This study aims to identify the features of clinical and immunological signs accompanying pathological changes caused by COVID-19 in moderate and severe diseases. To identify complications of acute respiratory disease caused by SARS-CoV-2, detected at autopsy. Retrospective analysis of the results of immunological and microbiological studies of 20 patients with confirmed SARS-CoV-2 infection who were treated in clinics of the city of Sumy in 2020-2021: Group 1: 25.0% with severe disease (mean age 62.1±2.1 years); Group 2: 75.0% with mild or asymptomatic disease (43.0±0.9 years). Analysis of 20 autopsy reports and inpatient medical records for deceased patients for the period from December 2020 to June 2021. The main morphological, clinical, and immunological changes detected in both groups are described, the changes in the body for acute respiratory viral infection SARS-CoV-2 in mild and severe forms of the disease are analyzed. The results of autopsies in severe COVID-19 that resulted in death are presented. The rapid spread of SARS-CoV-2 is caused by the antigenic variability of viruses under the influence of immune factors in the body of previously infected people and is associated with defects in the organization of medical care in medical institutions of the region and Ukraine as a whole, as well as with underfunding of the medical sector and health care.

Keywords: SARS-CoV-2, Immunology, Microbiology, Infection

Introduction

Problem Statement

The COVID-19 pandemic has become a global public health challenge, threatening millions of lives and dramatically changing our way of life (Agrati *et al*., 2022; Lv *et al*., 2022; Berre *et al*., 2023). The SARS-CoV-2 virus continues to infect people around the world, and the World Health Organization (WHO) has reported more than 436 million confirmed cases of COVID-19, including 5.95 million deaths (Jiménez & Torres Arias, 2022; Khorramdelazad *et al*., 2022). The pathogenesis is based on the damage to human cells mainly due to the direct cytopathogenic effect of the SARS-CoV-2 virus, as well as an overreaction of the immune system to the reproduction of the virus (Matthay *et al*., 2020; Feshchenko *et al*., 2021). The main manifestation of the disease is the defeat of various structures of the respiratory system with the development of primary viral pneumonia with its morphological and clinical features (Mathew *et al*., 2020; Patil & Jain, 2020; Rokade & Khandagale, 2020; Hariri *et al*., 2021; Talele *et al*., 2022). Severe respiratory failure in COVID-19 is caused by a specific pattern of immune disorders (Giamarellos-Bourboulis *et al*., 2020). Moderate to severe patients, especially among the elderly or those with comorbidities, are prone to deterioration, as evidenced by high mortality rates, but effective indicators of disease severity and therapeutic response have not yet been fully researched (Aldahlawi & Zaher, 2021; Khan *et al*., 2021). The problem of studying the mechanisms of the virus's effect on the human body, immune and pathogenetic changes that lead to severe disease, and significant mortality associated with SARS-CoV-2 infection remains extremely relevant (Suleimanova, *et al*., 2021).

Object of Study

Clinical condition, immunological parameters, and autopsy results in patients with moderate and severe SARS-CoV-2 infection

The Objective of the Study

1) to determine the features of clinical and immunological signs accompanying pathological changes caused by COVID-19 in moderate and severe disease; 2) to identify complications of acute respiratory disease caused by SARS-CoV-2, which are detected during autopsy. Viral diseases are a large group of infectious diseases diverse in clinical course, symptomatology, and morphology that are highly contagious and capable of causing epidemics and pandemics. The difficulty in fighting these diseases lies in the lightning-fast spread of the disease, active mutation of the virus, which entails the development of nonspecific clinical symptoms and multiple morphological changes in internal organs, as well as inefficient methods of diagnosis and treatment in existing protocols. Undoubtedly, Ukraine as a part of the global system is guided by the world experience in pandemic cases. Therefore, it is important to understand the cause-and-effect relationships when

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considering the most important issues in health care today. As of September 1, 2020, 25,327,098 people worldwide were diagnosed with COVID-19, according to World Health Organization (WHO) statistics1. The frame of our study was the period from September 2020 to June 2021. The chosen period is explained by the open access to the necessary materials: clinical, statistical, and pathologoanatomic data of patients and deceased patients. According to the laws of statistics, looking at a problem in a relatively small group may be the key to solving it on a larger scale over time. Currently, there is no single approved treatment for coronavirus infection, nor are there reliable data on patients suffering side effects of this treatment. The new clinical protocol of the Ukrainian Ministry of Health for the treatment of COVID-19 includes data based on the approval of the world's leading health authorities (including WHO), positive experience with diagnosis and treatment worldwide, and local protocols of many reputable clinics.

Materials and Methods

General Information

To fulfill the aim of the study, a retrospective analysis of the results of immunological and microbiological studies of 20 patients with confirmed SARS-CoV-2 infection who were treated in clinics of the city of Sumy in 2020-2021 was performed. The patients were divided into two groups based on the course of the disease (confirmation of the COVID-19 etiology was performed by a protocol-based RT-PCR test): 1) 5 patients (25.0%) with severe SARS-CoV-2 infection; 2) 15 patients (75.0%) with mild or asymptomatic disease. The average age of patients in group 1 was 62.1±2.1 years and in group 2 - 43.0±0.9 years. In the first group, all patients had a history of non-communicable diseases, namely diabetes mellitus, arterial hypertension, heart disease, and chronic kidney disease. All patients were examined according to the standard protocol for the diagnosis and treatment of COVID-19. The screening immunological tests included: complete blood count; CD-3+ cell count; phagocytic activity of blood phagocytes; serum M and G antibodies. Specific immunological studies included: determination of the total number of CD-4+ and CD-8+ cells; determination of IgM and IgG antibodies to SARS-CoV-2 coronavirus. All examinations were performed using generally accepted methods according to standard protocols. The results of the analysis of 20 autopsy reports and inpatient medical records for deceased patients for the period from December 2020 to June 2021 were also included in the study.

Data Analysis

The medical records were analyzed: clinical symptoms, disease features, data from additional examination methods (spiral computed tomography, X-ray, and ultrasound), and treatment results. Autopsy results were analyzed to identify pathomorphologic changes in internal organs and histologic findings. Obtaining, correcting, and systematizing the initial information and statistical analysis of the results were carried out using a licensed version of the spreadsheet processor Microsoft Office Excel 2019. The results were processed by descriptive statistics without comparing the described groups and checking the nature of the distribution. During our work, we analyzed the results of immunological and microbiological studies of 20 patients with a history of confirmed SARS-CoV-2 infection of varying degrees of severity (the final diagnosis was confirmed by RT-PCR):

Group 1 - 25% of patients with a severe course (age 62±2 yrs). These patients had a history of non-infectious diseases: diabetes, hypertension, heart disease, and chronic kidney disease.

Group 2 - 75% of patients (age 43±0.9 yrs) had a mild or asymptomatic course of infection.

This RT-PCR test was chosen as the main protocol diagnostic test according to the regulatory documents of the Ministry of Health of Ukraine and WHO.

It should be noted that as an additional study in patients with SARS-CoV-2 infection of mild and asymptomatic course of infection, serological examination by ELISA testing was used, which determines immunoglobulins of two types: IgM and IgG. In addition, the immune status (immunogram) of this group of patients was studied in the post-infection period using generally accepted methods11.

The immunogram included screening and confirmatory tests. Screening immunologic tests included:
1. total blood count;
2. total CD-3+ cell count;
3. phagocytic activity of blood phagocytes;
4. serum antibodies (M, G). Specific immunological examinations included: 1. determination of total CD-4+ cell count; 2. determination of total CD-8+ cell count;
3. IgM antibodies to SARS-CoV-2 coronavirus;
4. IgG antibodies to SARS-CoV-2 coronavirus.

The range of indices chosen for the immunological study was determined by the results of a literature review containing data on the immunopathogenesis of infection caused by SARS-CoV-2 coronavirus in patients with moderate to severe course.

Also, 20 autopsy reports and case histories of deceased patients were reviewed, of which: 6 were from October through December 2020 and 14 from January through June 2021. An analysis of the data was conducted: case histories: clinical symptoms; features of the course of the disease; methods of diagnosis (CT, X-ray studies) and treatment; autopsy: pathomorphological changes of internal organs; results of histological studies.

Literature Review

The scientific community around the world has made tremendous efforts to develop public health strategies, develop highly effective vaccines, and conduct advanced research on new treatments (Gu *et al*., 2022; Shafqat *et al*., 2022). The disease can have various clinical manifestations - from asymptomatic to severe critical conditions such as respiratory failure, septic shock, and multiple organ failure (Agrati *et al*., 2022; Cagan *et al*., 2022; Jiménez & Torres Arias, 2022). The pathogenicity of the SARS-CoV-2 virus is closely related to the immune status of a person (Fitriah *et al*., 2022; Gu *et al*., 2022), which is the first line of defense and plays a crucial role in the immunopathogenesis of COVID-19 syndrome

(Radandish *et al*., 2022); And while the immune system plays an important role in fighting pathogens, the virus can dysregulate the defense response and cause severe complications (Dewangan *et al*., 2020; Kumar *et al*., 2020; Jiménez & Torres Arias, 2022). The most prominent immune pathology of the progressive disease is a "cytokine storm" associated with a violation of the regulatory mechanisms of immune defense (Alahyari *et al*., 2022; Kusainov, 2022; Krasnova, 2022; Maltsev & Bokova, 2022). Failure to properly activate or eliminate inflammatory responses at certain stages can lead to disease progression, which means that a strong immune response to SARS-CoV-2 is crucial (Lv *et al*., 2022). The characterization of adaptive immune responses, including neutrophils, macrophages, CD4+ T lymphocytes, and B lymphocytes, plays an important role in resistance to infection (Jasim *et al*., 2022). For a detailed understanding of immune responses to infection, serologic testing is crucial to guide clinical therapeutic strategies (Baig *et al*., 2021; Berre *et al*., 2023). The T-cell immune responses associated with SARS-CoV-2 infection are still poorly understood, and the diversity of their repertoire is important for the formation of effective immunity (Bai *et al*., 2022; Cagan *et al*., 2022). Interferons, critical components of the immediate immune response to viral infections, can inhibit viral replication and spread. One of the available data (Cagan *et al*., 2022; Khorramdelazad *et al*., 2022) suggests that the antiviral response in patients with severe COVID-19 is impaired, and the use of exogenic interferon at different stages of the disease may lead to uncertain results (Agrawal *et al*., 2021; Bahramy *et al*., 2021). In the mild course of the disease, a higher frequency of highly differentiated T-lymphocytes was noted compared to healthy individuals, and in severe disease - an increased frequency of CD4+ memory effector T cells and highly differentiated CD8+ T cells and a decreased frequency of low-differentiated CD8+ T cells in the blood compared to patients with mild disease (Alahyari *et al*., 2022; Dorneles *et al*., 2022). That is, regulatory T cells may be one of the markers of disease severity and prognosis (Radandish *et al*., 2022). Patients with different COVID-19 severity have different levels of extracellular adenosine triphosphate and adenosine, as well as cytokines (Dorneles *et al*., 2022). The percentage of total CD4+ T-cells and NLRP3 gene expression, along with blood oxygen saturation and lactate dehydrogenase levels, have acceptable sensitivity and specificity for severe COVID-19 (Radandish *et al*., 2022). The expression of ectonucleotidase genes is negatively correlated with the level of C-reactive protein, an inflammatory marker of disease severity, and severely ill patients have higher plasma adenosine triphosphate and lower aspartate aminotransferase levels (Pietrobon *et al*., 2022; Navarro *et al*., 2023). When 280 participants from the University College of Antioquia (Colombia) were tested for SARS-CoV-2 antibodies in apical blood and plasma samples between November 2020 and January 2021 (Navarro *et al*., 2023), using standardized laboratory serological tests, it was found that 11.2% of people were positive for SARS-CoV-2 antibodies (IgG/IgM), of which 96.6% were in plasma samples and 37.9% in capillary blood samples. When comparing the two tests, the overall sensitivity and specificity of the capillary and plasma samples were 36.7% and 99.6%, respectively.

These studies have identified many pathogenic immune mechanisms of the disease, and vaccines against the virus have been successfully developed and applied in clinical practice. However, the pandemic is still ongoing and new mutations of SARS-CoV-2 are emerging, which threatens to make vaccines relatively limited in use (Al-Hamamy, 2022; Gu *et al*., 2022; Shafqat *et al*., 2022). Studying the interaction between the immune system and severe acute respiratory syndrome caused by SARS-CoV-2 is vital for the development of better clinical protocols, new therapeutics, antivirals, and vaccines) Studying the mechanisms of the immune response allows us to understand the immunopathogenesis of COVID-19 and identify its features that can be used for personalized treatment strategies (Jiménez & Torres Arias, 2022).

Results and Discussion

Morphological changes in the lungs in COVID-19 are manifold and are discussed in articles by researchers from many countries. Daily study of histological preparations of lung tissue gives new “findings” and makes us think how they could arise, and what terms to call them. In our opinion, new information about histological and immunological signs (markers) of COVID-19 course will change depending on the appearance of new variants of coronavirus strains, so not always existing markers can be characterized only by the terms “diffuse alveolar damage”, or “alveolar edema” or “atypical pneumonia”. First of all, the character of the course of infection may be related to individual characteristics of the immune status of a patient with COVID-19 and the level of pathogenicity of the COVID-19 strain. That is why it is reasonable and, in our opinion, eligible questions - whether different pathological changes can be forms or phases of the diverse process in lungs, or whether each new strain leads to the same pathomorphological and/or immunological shift in the organism, which can be used as a criterion of diagnosis. SARS-CoV-2 infection is known to have a variety of clinical manifestations, ranging from asymptomatic or mild infection to a severe course requiring hospitalization (Matthay *et al*., 2020). In people with asymptomatic or mild disease, little is known about protective immune responses, whereas hospitalized patients, often with severe pneumonia and acute respiratory distress syndrome (ARDS), may have either under-or overreacting immune cells with possible adverse outcomes (Matthay *et al*., 2020; Talele *et al*., 2022). One important sign of SARS-CoV-2 infection is lymphopenia (Matthay *et al*., 2020; Tan *et al*., 2020), which is associated with a severe course of the disease (Tan *et al*., 2020; Characterization of immune responses in COVID-19, 2022) and disappears when patients recover (Giamarellos-Bourboulis *et al*., 2020; Matthay *et al*., 2020). In some patients, lymphopenia affected CD4+-, CD8+-, B-cells, and NK-cells (Mathew *et al*., 2020; Matthay *et al*., 2020) whereas many other works suggest that the SARS-CoV-2 virus preferentially affects CD8+-T cells (Mathew *et al*., 2020; Matthay *et al*., 2020; Hariri *et al*., 2021). Temporary lymphopenia is known to be a common feature of many respiratory viral infections, such as those caused by influenza A3H2 virus, human rhinovirus, or respiratory syncytial virus, but in the case of these infections, lymphopenia usually lasts only 2-4 days after the onset of symptoms and rapidly (Matthay *et al*., 2020). Several immunological features have been described that

are characteristic of patients with severe COVID-19: high levels of systemic cytokines or chemokines, in particular IL-6, CXCL8, CXCL9, and CXCL10, impaired induction and synthesis of interferon (INF) type I. All of these can affect the T-cell link of the immune response. Comorbidities such as cardiovascular disease, diabetes, and obesity that cause a severe, complicated course of COVID-19 may also affect the activity of the T-cell immune response. How comorbidities affect immune responses in this infection is not fully understood, although there are several conflicting explanations for this phenomenon (Fitriah *et al*., 2022; Matthay *et al*., 2020; Talele *et al*., 2022).

The analysis of ELISA tests for the detection of IgM and IgG antibodies to SARS-CoV-2 coronavirus revealed the level of these antibodies at the time of infection and in the post-infection period (4.5 ± 0.7 months) was different **(Table 1)**.

Table 1. Dynamics of anti-SARS-CoV-2 antibody detection in patients with mild and asymptomatic course

Indicator	Result	Units	Reference group indicators
Leukocytes	9,9±0,6	$\times 10^9$/l	4,0 – 9,0
Mononuclear	1096±1,1	abs. number	1200 - 3000
T-lymphocytes (CD2CD3)	635±3,2	abs. number	720 - 2000
CD8-lymphocytes	184±1,6	abs. number	200 - 400
CD4-lymphocytes	367±2,7	abs. number	400 - 800
CD22, CD19-lymphocytes	197±1,5	abs. number	200 - 400
CD16-lymphocytes	15	%	16 - 22
Plasma C-reactive protein	9	Mg/l	≤ 10

Source: authors' development

Immunogram analysis of patients with asymptomatic SARS-CoV-2 infection and reinfection with this virus after 9 ± 0.3 months showed leukocytosis with slight lymphopenia and CD4+-, CD8+-cell deficiency **(Table 2)**.

Table 2. Results of immunological examination of patients with a history of asymptomatic SARS-CoV-2 infection in the post-coV period (4.5±0.7 months)

anti-SARS-CoV-2 antibodies	Antibody concentrations in patients with SARS-CoV-2 infection		Antibody concentrations in patients in the post-covid period	
	asymptomatic course	light current	asymptomatic course	light current
IgM	1,97±0,6	12,83±0,9	-	-
IgG	6,72±0,7	15,63±0,7	4,43±0,6	5,81±0,6

Source: authors' development

Based on the results of the detection of virus-specific IgM, IgG we have found that the titer of highly specific anti-SARS-CoV-2 IgG in patients with asymptomatic and mild course decreases significantly in the remote post-Covid period (4.5±0.7 months.).

The humoral immune response is thought to be crucial for the elimination of cytopathic viruses from the body and is a major part of immune memory, which prevents reinfection. SARS-CoV-2 leads to a persistent B-cell response, as evidenced by the rapid and nearly universal detection of virus-specific IgM-, IgG- and IgA-antibodies as well as neutralizing IgG-class antibodies in the first 7-10 days after infection. The kinetics of the humoral response to SARS-CoV-2 have been well-studied (Matthay *et al*., 2020). On the other hand, the concentration of virus-specific antibodies in patients with asymptomatic SARS-CoV-2 infection is significantly ($p \geq 0.5$) lower than in patients with mild infection **(Table 1)**. For this reason, it cannot be argued that the asymptomatic course of SARS-CoV-2 infection can provide a meaningful effective long-term concentration of anti-SARS-CoV-2 antibodies that bind internal protein N and external glycoprotein SARS-CoV-2.

Long-term protection against SARS-CoV-2 is achieved through the induction of plasma cells and the formation of a pool of memory B-cells (Matthay *et al*., 2020), but low antibody levels in asymptomatic individuals may indicate the formation of so many plasma cells and the formation of a pool of memory B-cells that do not provide long-term protection against SARS-CoV-2. This assumption is confirmed by the fact that 71.4% of patients examined who had a history of asymptomatic SARS-CoV-2 infection were reinfected with this virus after 9±0.3 months with a moderate course of infection. Laboratory findings in patients with SARS-CoV-2 infection are nonspecific but usually include leukocytosis with lymphopenia, moderate increases in liver transaminases, muscle enzymes, myoglobin, and LDH, and elevated markers of the acute phase of inflammation. Increased procalcitonin concentration, severe lymphopenia, and elevated D-dimer levels are signs that accompany a more severe course of the disease. In severe cases, the disease can progress to respiratory, cardiovascular, and renal failure and finally to death from multiple organ failure (Kondody *et al*., 2022; Talele *et al*., 2022; Coronavirus infection lecture, 2023).

It should be noted that 28.6±0.3% of this category of examinees had cytomegalovirus IgG (14.11±0.2 S/CO) and immunogram values different from those obtained from patients who had been reinfected with SARS-CoV-2 infection **(Table 3)**.

Table 3. Results of an immunologic study of patients in the post-Covid period (4.5 ± 0.7 months) who had a history of asymptomatic SARS-CoV-2 infection and had no reinfection

Indicator	Result	Units	Reference group indicators
Leukocytes	6,85	$\times 10^9$/l	4,0 – 9,0
Mononuclear	1672	abs. number	1200 - 3000
T-lymphocytes (CD2CD3)	873±3,2	abs. number	720 - 2000
CD8-lymphocytes	668±1,6	abs. number	200 - 400
CD4-lymphocytes	942±2,7	abs. number	400 - 800
CD22, CD19-lymphocytes	245±1,5	abs. number	200 - 400
CD16-lymphocytes	14	%	16 – 22
Plasma C-reactive protein	7	mg/l	≤ 10

Analyzing the immunogram of people with a history of asymptomatic SARS-CoV-2 infection, we identified signs of prolonged post-infection secondary immunodeficiency: leukocytosis with lymphopenia, and CD4+-, CD8+-cell deficiency.

According to the literature, 5-7 days after the onset of SARS-CoV-2 infection, interstitial pneumonia occurs, initially focal and quickly transforming into confluent pneumonia. The mononuclear phagocyte system is affected; lymphopenia develops, and IFN synthesis is depressed. Coronavirus pneumonia can be complicated by the accession of bacterial flora, as evidenced by increased serum procalcitonin levels. It also occurs when the patient's condition worsens. In addition, the severity of the disease is indicated by high levels of C-reactive protein (CRP), lactate dehydrogenase (LDH), D-dimer, ferritin, etc. In parallel, there are changes in the clotting system. Hemoglobin level decreases, which aggravates hypoxic syndrome (Andreychyn *et al*., 2020; Talele *et al*., 2022). According to WHO guidelines, antibiotic therapy may be indicated for the treatment of severe SARS-CoV-2 infection. Antimicrobial therapy should be used in patients with COVID-19 only if there is a confirmed bacterial co-infection (i.e., after a positive bacteriological blood test and/or sputum) since antibiotics do not affect the virus. Amoxicillin/clavulanic acid, respiratory fluoroquinolones, 3rd and 4th generation cephalosporins, carbapenems, linezolid, etc. are used more often (Detrick *et al*., 2016; Gupta, 2020; National Institute for Health and Care Excellence, 2023). Given the fact that 25% of patients in Group 1, who had a severe SARS-CoV-2 infection (age 62±2 yrs), used empirical antibiotic therapy with broad-spectrum drugs, we analyzed the adhesive properties (as a pathogenicity factor initiating the development of infectious disease) and antibiotic resistance of clinical isolates of bacterial opportunistic pathogenic upper respiratory tract microflora of this category of people during the post-infection period (4.5±0.7 months).

In the analysis of microbiological examination in the composition of the upper respiratory tract microflora of this category of people were found highly adhesive strains of *Klebsiella pneumoniae* and *Staphylococcus aureus* (microorganism adhesion index (IAM) ≥ 9.2 bact./er) and *Streptococcus pneumoniae*, which 6.2 bact./er); highly adherent strains of *Candida spp.* Representatives of the upper respiratory tract bacterial normoflora had different antibiotic sensitivity profiles: 100% of *Staphylococcus aureus* strains were resistant to amoxicillin, 50% to respiratory fluoroquinolones (levofloxacin), 100% to. 100% of clinical isolates of *Klebsiella pneumoniae* showed resistance to levofloxacin and 3rd generation cephalosporins.

Due to the fact that against the background of severe course of SARS-CoV-2 pneumonia complicated by bacterial co-infection, antibiotic therapy is prolonged, it should be taken into account the fact that the microflora of many human biolocuses includes *Candida spp.* This is due to the fact that prolonged antibiotic therapy inhibits normal microflora and promotes increased adhesion of fungi and their colonization of the epithelium, including the upper and lower respiratory tract. According to the results of our study of the microflora of patients with a history of a severe course of SARS-CoV-2 pneumonia and antibiotic therapy, highly adherent *Candida spp.* and lymphopenia with deficiency of CD4+, CD8+ cells were found in the post-covid period. All above-stated argue for the expediency of the application of antifungal antimycoprophylaxis (based on results of individual sensitivity of isolated strains to antifungal drugs) of complications of fungal etiology in the given category of patients.

Twenty autopsy reports and case histories were reviewed, of which: 6 were from October through December 2020 and 14 from January through June 2021.

The average age of the deceased in 2020. - Men 86-87 years, women 78-79 years; in 2021 - men 68-69 years, women 60-61 years.

The following patients died at home: in 2020. - 4 patients; in 2021 - 2 patients.

Average time of death: in 2020. - 02.00 to 11.30; in 2021, 18 patients from 02.00 to 09.16, and 6 patients died from 16.40 to 23.30.

The average duration of illness: in 2020. - 10 days; in 2021: men - 7-8 days; women - 9-10 days. Liquidators at Chernobyl NPP: in 2020. - 0; in 2021 - 2 people.

In 23 cases, the main disease: Acute respiratory disease caused by SARS CoV 2 (virological study data: SARS CoV 2 virus, real-time PCR method; positive result): acute serous tracheobronchitis/bronchiolitis with desquamation and squamous cell metamorphosis pneumonia, acute respiratory distress syndrome.

One case of the combined underlying disease:

1. Acute respiratory disease caused by SARS CoV 2 (virological study data: SARS CoV 2 virus, real-time PCR method; positive result): acute serous tracheobronchitis/bronchiolitis with desquamation and flat-clotting total hemorrhagic pneumonia; acute respiratory distress syndrome with manifestations of carnification.
2. Coronary heart disease: acute small focal posterior-lower myocardial infarction.

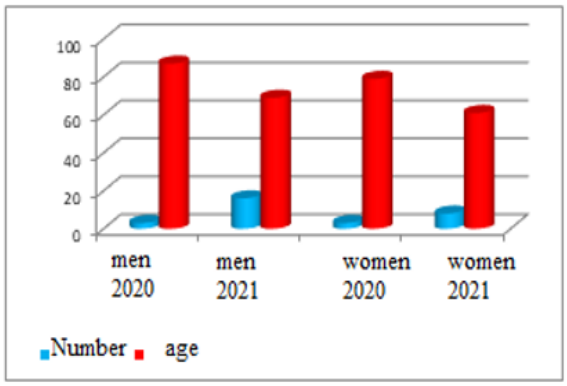


Figure 1. Quantitative Indicators of statistical deaths in 2020-2021

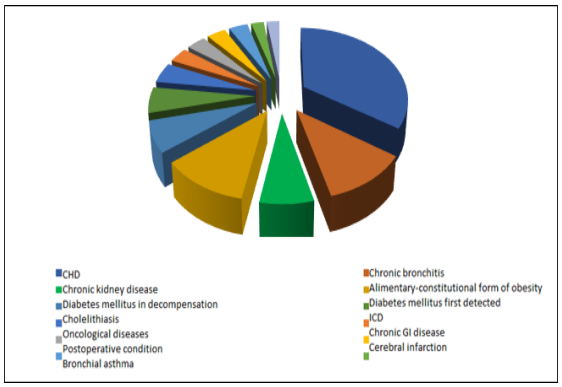
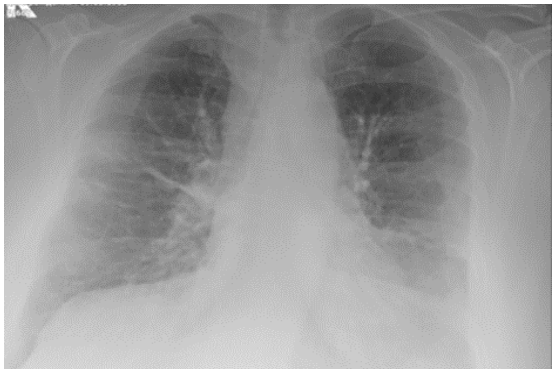


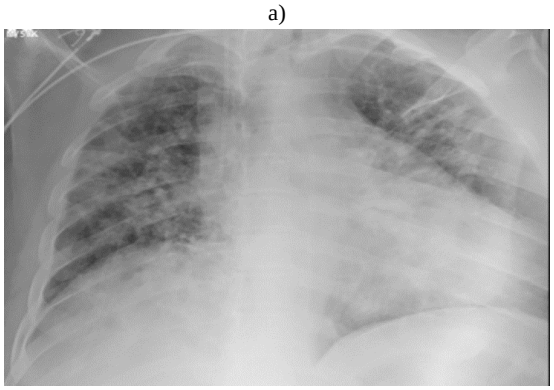
Figure 2. Types of concomitant pathology in the deceased in 2020-2021

In general, the complications were as follows: acute pulmonary-cardiac failure. Pulmonary edema. Oedema of the brain. General intoxication. Tension right-sided pneumothorax. Condition after drainage of the right pleural cavity. Thromboembolism of the main trunk of the pulmonary artery (thromboembolism of small branches of the pulmonary artery). Generalized microcirculatory disorders. Bilateral exudative pleurisy. Parenchymatous dystrophy of internal organs. Erosive gastro-duodenitis. Fatty dystrophy of the liver.

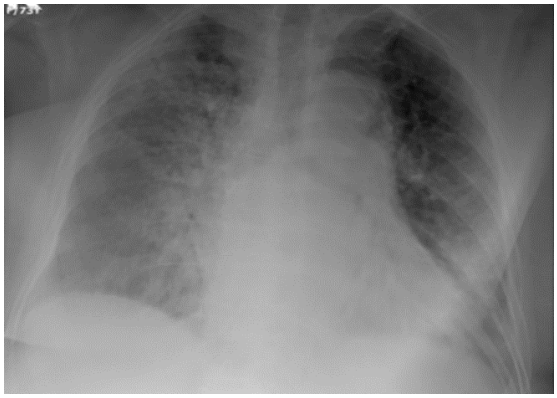
X-ray and CT scans demonstrated various degrees of pulmonary tissue damage, which were associated with clinical signs of pulmonary insufficiency (see supplementary materials).



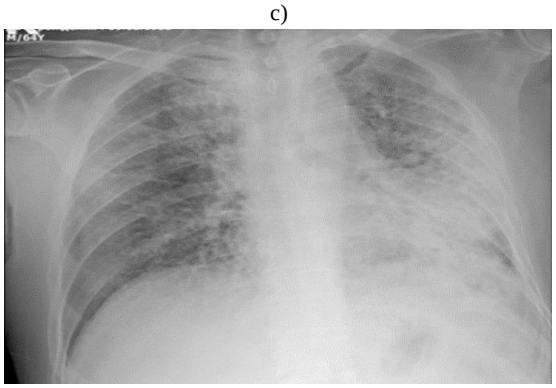
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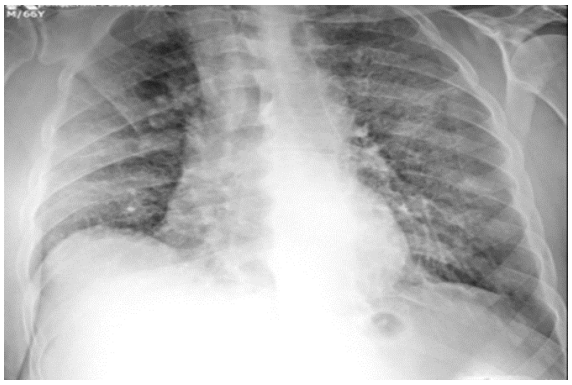
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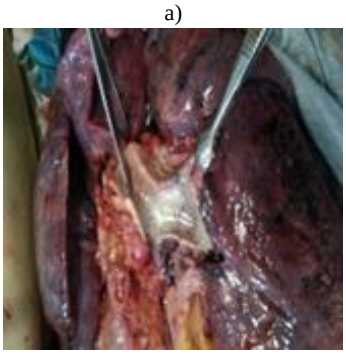


e)

Figure 3. a) X-ray of diffuse interstitial lung changes. b) X-ray showing bilateral polysegmental interstitial pneumonia. Hypertrophy of the heart. c) X-ray showing bilateral poly segmental interstitial pneumonia. Interstitial edema on the right side. d) X-ray showing bilateral poly segmental interstitial pneumonia. Interstitial edema on the left side. e) X-ray showing bilateral multisegmental interstitial pneumonia.



a)



b)



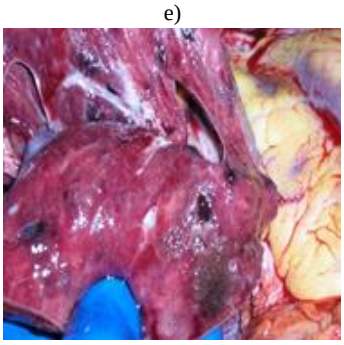
c)



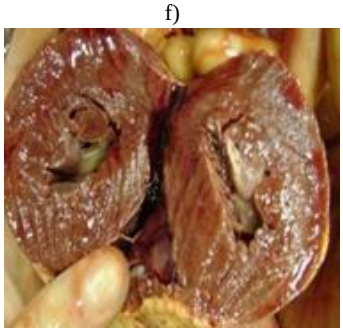
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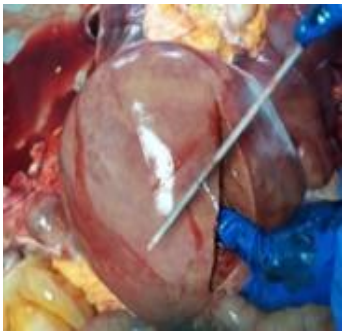
e)



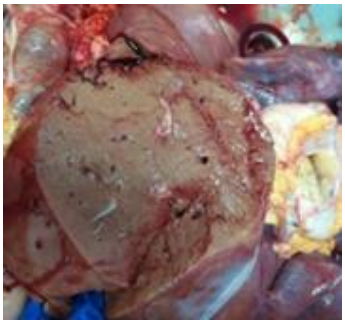
f)



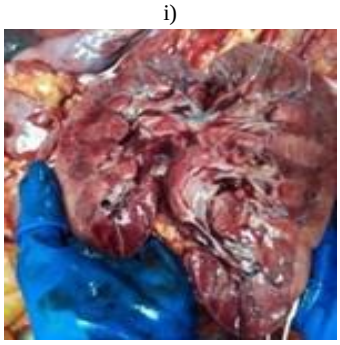
g)



h)



i)



j)

Figure 4. a) Hemorrhages in the mucosa of the trachea and bronchi. b) Pulmonary edema (frothy fluid in tracheal duct). c) Subpleural foci of pulmonary hemorrhages. d) The lungs are enlarged in volume, reddish-burgundy in color, sparsely airy with foci of hemorrhages. e) Thrombi of the pulmonary artery. f) Pulmonary apoplexy due to thromboembolism of small branches of the pulmonary artery. g) Myocardial hypertrophy. h) Diffuse hemorrhages under the liver capsule. i) Parenchymatous dystrophy of the liver. j) Renal venous pleural congestion.

Data from Medical Records

At referral to family physicians and hospitalization, patients presented the following complaints: general weakness; dry cough; difficulty in breathing (dyspnea during loading and at rest); temperature increase from +37°C to +38°C; dry rales (mainly in the lower lungs); respiratory failure. SpO2 saturation by the time of referral and hospitalization was: from 95% to 53%, which averaged 82-83%.

In clinical blood tests, there was a decrease in hemoglobin to an average of 118-119 g/l, an increase in erythrocyte sedimentation rate (ESR) to an average of 38-39 mm/h; an increase in sugar to an average of 11.7-12.0 mmol/l; white blood cell and red blood cell levels were in the normal range: 6.9-7.0x109, 3.5-4.0x10^{12}.

Regarding the indices of biochemical blood analysis, total protein averaged 4.0 g/l; in most analyses, urea and creatinine levels were elevated from 3.8 to 82.4 mmol/l, respectively, with an average of 17.73 mmol/l urea and 78 to 120 µmol/l creatinine; total bilirubin averaged 18.1 µmol/l, which is normal. The clinical urinalysis shows elevated levels of protein averaging 0.516 g/l and sugar 0-2 mmol/l and in some cases up to critical levels of 55 mmol/l. We present a typical example of an autopsy protocol (all supplementary materials are available from the authors).

The trachea and bronchi show pink mucosa with hemorrhages and mucus. Frothy red fluid is present in the tracheal lumen. Pleural cavities contain approximately 200-300 ml of clear fluid, with smooth, shiny pleural leaves filled with blood. Irregularly shaped hemorrhages are observed under the visceral pleura. The lungs appear enlarged, reddish-burgundy, and dense. Dark red lung tissue with scattered hemorrhage foci is observed upon sectioning. Abundant red foamy liquid flows from the sectioned surface. The bronchial mucosa is shiny, smooth, bright pink, with hemorrhages and mucus. In some cases, the lungs are enlarged, reddish-burgundy, and dense, with collapsed volume up to 2/3. Dark red thrombi are found in the pulmonary arteries, forming "columns," along with lung infarcts in the shape of dark red triangles. Lymph nodes near the trachea and bronchi are enlarged, pale pink, and dense. The heart appears enlarged, with clay-like, flabby myocardium. The left ventricular wall is thickened, dark red, and shows gray fibrous layers. The pericardial cavity contains clear or yellow fluid with fibrin. Coronary arteries show irregular narrowing due to atherosclerotic plaques. The liver is slightly enlarged, with a smooth surface and brown-yellow parenchyma. The kidneys have clear borders, dark-red parenchyma, and easily detachable capsules. Cerebral base vessels are thickened, and transparent serous fluid is slightly increased in the ventricles. Virological examination confirms positive SARS-CoV-2 virus detection in all cases using real-time PCR method. Due to the fact that against the background of severe course of SARS-CoV-2 pneumonia complicated by bacterial co-infection, antibiotic therapy is prolonged, it should be taken into account the fact that the microflora of many human biolocuses includes Candida spp.

In our study of patients with a severe course of SARS-CoV-2 pneumonia and antibiotic therapy, we found highly adherent Candida spp. and lymphopenia with CD4+ and CD8+ cell deficiency in the post-COVID period. These findings suggest the need for antifungal antimycotic prophylaxis based on individual strain sensitivity to antifungal drugs in these patients. Analysis of case histories and autopsy reports revealed that late-stage SARS-CoV-2-induced respiratory illness was characterized by general weakness, increased fatigue, pronounced intoxication, elevated body temperature, inflammation signs in clinical and biochemical tests, and histological changes in sectional material. Patients exhibited a dry cough with moderate catarrhal symptoms and experienced rapid development of complications involving internal organs and increased airway and lung damage. Many patients and deceased individuals initially experienced mild cold-like symptoms, leading to delayed medical care and refusal of prophylactic vaccination. In comparison to the situation in 2019, there has been a significant increase in mild, sterile, and asymptomatic forms of acute respiratory viral infections. The patients in our study were elderly (60 to 89 years old) with a history of chronic diseases, which contributed to periodic exacerbations and immunodeficiency. The characteristic clinical signs of acute respiratory disease caused by SARS-CoV-2 became pronounced only in the extremely severe cases.

Conclusion

Analyzing the data from research publications, case histories, and autopsy reports, life-threatening complications are exactly the

following: acute pulmonary heart failure; pulmonary edema; cerebral edema; intoxication; thromboembolism of the main pulmonary artery trunk; thromboembolism of small pulmonary artery branches with lung infarcts; generalized disorders of microcirculation; alternative changes of internal organs: necrotic nephrosis; centrilobular necrosis of hepatocytes; necrosis of endocrine cells of the pancreas complications, in turn, developing at the expense of sterile clinical symptomatology due to untimely initiated specific therapy. Difficulties in early diagnosis of acute respiratory disease caused by SARS CV 2 are primarily due to the fact that patients themselves were negligent about their health condition, they turned to family doctors late, engaged in self-medication (took antipyretic, antibacterial, antiviral drugs), medical history, body temperature indicators at the time of examination and hospitalization within - +37-38°C; sterility of specific symptoms due to the older age of patients and the presence of a large number of chronic comorbidities. The high mortality rate of patients at home in 2019-2020 can be explained by the absence, at that time, of certain schemes for the standards of diagnosis, treatment, and rehabilitation of patients, both at the primary and secondary levels of medical and preventive care. Appointment of ineffective antiviral therapy by family doctors; use of antipyretic drugs for subfibrile body temperature; keeping patients in primary care for a long time; late additional methods of investigation such as lung X-ray, CT; prescription of massive doses of different antibacterial drugs. But this in 2019-2020, primarily contributed to the lack of scientific and practical data on the effect of this type of virus on the body, which led to the ineffectiveness of the treatments used. And also, an important role was played by the anti-advertising of traditional medicine, the unreliability of the information promulgated in the media, “rail” information in social networks, causing panic, the indignation of the population, and increased distrust of society towards doctors and medicine in general, especially in small towns and villages.

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