Danylo Halytsky Lviv National Medical University
Department of Clinical Immunology and Allergology

METHODOLOGICAL DEVELOPMENT OF TOPIC №7

practical lesson **"Immune-dependent post-COVID complications"** (2 hours) in the discipline "Clinical Immunology and Allergology" for 6-th year students of specialty "Medicine"

Theme №8 "Immune-dependent post-COVID complications"

1. **Background:**

Actuality: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a new member of the coronavirus family that can cause coronavirus disease 2019 (COVID-19). COVID-9 has become a global pandemic with severe health issues around the world. Identifying the accurate immunopathogenesis of the COVID-19 and the immune response against SARS-CoV-2 is necessary for the development of therapeutic approaches and rational drug design. Today it is especially important for students to understand the importance of knowledge on the diagnosis, treatment and prevention of viral infection COVID-19

2. **Aim:**

Academic – students need to know the clinical, instrumental and laboratory diagnosis of COVID-19, the main approaches to its treatment.

Professionally-oriented - students should be able to establish indications for the use of immunotropic therapy eo avoid COVID-19 complications.

Educational - students need to have a sense of responsibility for the timeliness and correctness of professional actions.

3. **Materials:** Equipment to run powerpoint presentation
   Main books. Short information due to the topic.
4. **Interdiscipline integration**

Subjects	To know	To be able to
Histology and embryology	Histological changes in lung tissue in patients with COVID-19	Be able to determine the inflammatory process in the tissue according to the morphological picture
Medical biochemistry	Basic methods of isolation and purification of proteins with antigenic properties	To choose methods of biochemical purification to isolate protein preparations from blood plasma
Therapy	Diagnostic criteria for SARS-CoV-2 virus infection	To diagnose the severity of COVID-19, establish indications for the use of biological therapy
Pediatrics	SARS-CoV-2 infection in children	To determine indications for the use of biological therapy for the treatment of COVID-19 in children
Molecular biology	Basic biotechnologies (hybridoma, recombinant)	Using of modern biotechnologies in the production of vaccines for the prevention of COVID-19

5. Study questions.:

1. Epidemiology, structure of SARS-CoV-2 virus infection
2. Pathogenesis of COVID-19
3. Symptoms of COVID-19
4. COVID-19 treatment
5. COVID-19 complications

Main part

The SARS-CoV-2 virus infects humans via droplets, and to some extent, aerosols. In symptomatic adults, the disease typically presents after 2–14 days of incubation as a respiratory illness with fever, cough, headache, myalgia and in some cases intestinal symptoms. A growing number of studies are pointing toward asymptomatic infection in a significant fraction of individuals, and as many as half of all transmission events occur from presymptomatic and asymptomatic individuals.

In this Perspective, I discuss what we know about the immune response to SARS-CoV-2 infection and how this might explain different disease presentations and disease severity by considering known immunological differences between the groups that are most commonly affected.

COVID-19 disease courses

Mild and severe acute COVID-19

It is clear that the outcome of infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) varies broadly, with the majority of young individuals experiencing mild disease. Also, sex is an important; men are over-represented among patients with severe disease, presumably due to differences in the elicited immune responses. Comorbidities such as obesity, hypertensive disease, chronic obstructive pulmonary disease and cardiovascular disease are all associated with severe COVID-19 disease. Higher SARS-CoV-2 copy numbers at diagnosis have been reported in patients with severe COVID-19 than in those with mild COVID-19. Smoking is yet another risk factor: cigarette smoke induces expression of angiotensin-converting enzyme 2 (ACE2), which allows SARS-CoV-2 to enter cells, and could possibly influence viral invasion beyond its negative effects on overall lung function.

Despite the increased risk of severe disease with increased age, a minor subset of young and middle-aged individuals present with severe COVID-19 disease characterized by poor oxygen saturation and massive inflammatory responses in the lung. Such cases need urgent management and intensive care, and several studies have attempted to unravel the mediators of such hyperinflammatory disease presentation.

Long COVID

Apart from the differences in severity among patients with acute COVID-19, it is now clear that a number of other outcomes are possible after an initial infection with SARS-CoV-2.

After a long period of intensive care and mechanical ventilation, general anesthesia and severe illness, it is not surprising that long rehabilitation periods are needed. However, it is now also clear that some individuals with milder initial symptoms of COVID-19 can suffer from variable and debilitating symptoms for many months after the initial infection. This condition is popularly referred to as long COVID. An exact definition is lacking, but typically symptoms with a duration >2 months are considered long COVID. The condition involves a range of symptoms such as persistent fatigue, myalgia, autonomic dysregulation manifested as postural orthostatic tachycardia syndrome, abnormal thermoregulation, intestinal disturbances and skin manifestations. This post-COVID syndrome bears resemblance to postinfectious syndromes that followed outbreaks of chikungunya and Ebola, for example, and selected symptoms overlap with myalgic encephalomyelitis, a disease that is also often triggered by infection and immune activation and manifests as a dysregulated autonomic nervous system and perturbed immune parameters. More research is needed to understand the pathogenesis of all of these postinfectious conditions, and long COVID offers a unique opportunity to perform such studies in larger numbers of individuals, all infected by the same virus during a limited time frame.

Multisystem inflammatory syndrome associated with COVID-19

Another rare and serious postinfectious condition that can occur 2–6 weeks after SARS-CoV-2 infection is the multisystem inflammatory syndrome associated with COVID-19, first described in children (MIS-C) , and more recently in young adults (MIS-A). This hyperinflammatory syndrome shares clinical features with Kawasaki disease, but affects children who are older than the typical patient with Kawasaki disease and who more often present with intestinal involvement and myocardial failure and shock. There is also significant clinical overlap in presentation with toxic shock syndrome or septic shock. Subgroups of children affected by MIS-C are being described, and optimal management is being worked out by collaborative networks of pediatricians. Most MIS-C patients are treated with strong immunomodulatory regimens such as high-dose steroids, intravenous immunoglobulins and anti-cytokine therapies coupled with anti-coagulation to counter the microangiopathy and activation of both complement and coagulation cascades during the hyperinflammatory disease phase. The pathogenesis of MIS-C is unknown, but a delay of 2–6 weeks from initial SARS-CoV-2 infection indicates a role for adaptive immune responses and specific autoantibodies have been proposed.

Viral recognition and innate immune responses

Viral entry

SARs-CoV-2 infects cells by attaching to the principal viral entry receptor, ACE2. The expression of this receptor has been reported in single-cell messenger-RNA-sequencing data on epithelial cells in the oral mucosa, liver, kidney, intestine and heart, and at the protein level in alveolar epithelial cells, although the tissue distribution of protein expression differs to some extent. Several reports have shown abundant expression of ACE2 in the intestinal epithelium leading to viral shedding via feces, while ACE2 does not seem to be expressed by cells of the immune system.

Innate immune responses

SARS-CoV-2, like the related SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), is a single-stranded RNA virus. After entering a target cell, the virus is recognized by pattern recognition receptors such as Toll-like receptors 3, 7, 8 and 9 and viral-infection sensors RIG-I and MDA5, and viral recognition induces the type I interferon (IFN) response program and IFN-stimulated genes. The TLR3 response triggers transcription of the NLR family pyrin domain containing 3 (*NLRP3*) gene, which together with other cellular responses to viral infection—such as the formation of reactive oxidative species, calcium flux from cytoplasmic storages, protein aggregation and the release of danger-associated patterns—contributes to the activation of the NLRP3 inflamasome and likely other inflammasome complexes. The NLRP3 inflammasome induces caspase-1-dependent cleavage and release of key proinflammatory cytokines interleukin-1β (IL-1β) and IL-18, and triggers gasdermin D-mediated pyroptotic cell death. The extent of NLRP3 activation correlates with COVID-19 disease severity. As a result of pyroptotic cell death, the enzyme lactate dehydrogenase (LDH) is released. Elevated LDH levels have been observed in the blood of patients with COVID-19, and levels of this enzyme correlate with disease severity. Together, these data suggest that inflammasome activation is an important feature of COVID-19. This pathway also triggers the coagulation cascade, for example via the extracellular release of gasdermin D, and coagulopath and severe thrombotic events are common in patients with severe COVID-19. A similar activation of the coagulation cascade and elevated LDH levels are also seen in patients with MIS-C, but not in patients with long COVID, indicating differences in the underlying pathogenesis.

A characteristic feature of SARS-CoV and MERS-CoV viruses is their ability to inhibit and delay the induction of type I IFN by infected cells, which contributes to the immunopathology associated with such infections. Also, SARS-CoV-2 is able to inhibit the type I IFN responses in infected cells, leading to delayed or overall suppressed type I IFN responses. This allows the virus to replicate and induce more tissue damage, and triggers a more exuberant immune response as the immune system struggles to limit viral replication and to manage dying and dead cells. Immune pathology continues as inflammatory cells flow into the lung and produce large amounts of proinflammatory cytokines, further escalating the situation. Such imbalanced immune responses, caused in part by the impaired early type I IFN responses, are the most likely determinant of the overall severity of acute COVID-19 (This is further emphasized by recent results from the COVID Human Genetic Effortwhich found that inborn errors in the type I IFN pathway, or the presence of neutralizing autoantibodies to type I IFNs, were strongly over-represented among individuals who developed life-threatening COVID-19. Whether imbalanced or impaired innate responses also contribute to the development of other disease manifestations such as MIS-C and long COVID remains to be determined.

Adaptive immune responses

Serological tests for SARS-CoV-2 have been the subject of much discussion and conflicting results during the course of this pandemic so far. However, with time it has become apparent that the adaptive immune responses induced by SARS-CoV-2 infection largely follow the expected patterns based on what is known from other comparable viral infections, with >90% of infected individuals seroconverting a few weeks after initial infection. Presence of anti-spike IgG antibodies were associated with protection from reinfection in a UK cohort of health-care workers at high risk of exposure.

T cell responses to the SARS-CoV-2 spike protein correlate with B cell responses to the same protein and are detectable in nearly all convalescent patients with COVID-19. T cell reactivity to SARS-CoV-2 can also be detected in unexposed individuals, presumably due to cross-reactive immunity to common-cold coronaviruses or to other antigens, as has been shown for other virus-specific T cells. Another study has reported SARS-CoV-2-reactive T cells in patients who survived the SARS epidemic in 2003, but also in unexposed individuals; interestingly, such responses preferentially targeted epitopes different from the ones in convalescent patients with COVID-19, and were not homologous with common-cold coronaviruses, but conserved among animal coronaviruses.

Antibody-dependent enhancement (ADE), a phenomenon that has been described for infections with viruses such as dengue, has been proposed as a possible mechanism of severe COVID-19. ADE occurs when antibodies target a virus without neutralizing it, for example if the antibody is raised against a different serotype of the virus or when the antibody fails to block viral entry. Then, the antibody might facilitate Fc-receptor-mediated endocytosis of the virus and enhanced viral replication, and massive inflammatory responses. This has been described to occur for MERS, but no clear evidence of ADE as a cause of severe SARS-CoV-2 infection has been communicated. Reinfections have been reported, and in a few instances, the second infection was more severe than the first, but serological responses suggest that patients never seroconverted after initial infection and ADE is a less likely cause of a more severe second infection.

The role of pre-existing immunity to common-cold coronaviruses is another possible determinant of COVID-19 disease severity. T cell reactivity is found in unexposed individuals and has been linked to prior exposures to common-cold coronaviruses. Also, IgG that is specific to SARS-CoV-2 spike protein has been found in unexposed individuals, particularly in children and young adults, and some of these had neutralizing activity against SARS-CoV-2, indicating a potentially protective effect against severe COVID-19. Another study also identified such antibodies but found no evidence for a protective effect against COVID-19. Cross-reactive antibodies are also more frequently found in serum samples collected in sub-Saharan Africa prior to the COVID-19 pandemic, indicating a possible explanation for the surprisingly low number of severe COVID-19 cases seen on this continent. Whether there is a role for cross-reactive antibodies or T cells, or the absence of such features, in determining other disease manifestations, such as MIS-C or long COVID, remains to be seen. Children who develop MIS-C have detectable IgG responses without obvious differences from convalescent children without MIS-C, although one study indicated subtle IgG-subclass and functional differences between children with MIS-C and those without MIS-C.

Known immunological differences between high- and low-risk individuals

The risk of developing severe COVID-19 increases steeply above age 70, and also with the severity of obesity and other risk factors. Men have a much greater risk of severe acute COVID-19 than women, whereas women are over-represented among patients who develop long COVID. The infection differs from many other respiratory infections in that children are seemingly able to cope, even in the very first years of life, without developing severe

respiratory disease except in a few rare cases. The known immunological differences between young and old people and between men and women should help us further unravel the immunological mechanisms behind disease presentation and severity.

Sex differences

As mentioned above, type I IFN responses are critical determinants of disease severity during acute SARS-CoV-2 infection, and the virus has developed methods for subverting these responses. Women elicit stronger type I IFN responses upon stimulation with TLR7 ligandsand develop stronger vaccine responses, but also more side effects, and have better survival rates for a number of acute infections than do men. These sex differences are seen even in boys and girls before puberty—pointing towards genetic, rather than hormonal, differences. It is worth noting that a common sensor of viruses, TLR7, is expressed on the X chromosome, providing a possible difference in gene-dosage effect between men and women. Interestingly, the neutralizing autoantibodies to type I IFN found in patients with severe COVID-19 were much more abundant in men than in women, but the reasons for this are elusive. Immune-response differences have also been reported between male and female patients with COVID-19, and collectively these sex differences could explain the overall susceptibility of male patients to developing severe acute COVID-19. MIS-C is quite evenly distributed between boys and girls, whereas long COVID is more prevalent in female patients. It is also important to consider whether social factors and differing exposure play a part in sex differences.

Age differences

If type I IFN responses were the sole determinants of COVID-19 severity, one would expect young children to be highly susceptible because both newborn and young children produce lower amounts of type I IFN upon stimulation through various viral-sensing pathways. The low risk of severe SARS-CoV-2 disease in young children also differs from that of other respiratory viral infections like the flu, and points toward other protective mechanisms in young children. The immune systems of young children are accustomed to facing novel challenges, whereas older individuals rely more on memory responses. The thymus decreases its output of naive T cells and involutes at a rate of about 3% per year, and some data indicate more rapid involution in boys than in girls. Cross-reactive antibodies to common-cold coronaviruses are one possible explanation; another possibility is that constitutive differences in immune system states between young and old people are of importance.

Disease severity in COVID-19 also correlates with neutrophil-to-lymphocyte ratio (NLR), a metric reflecting immune-cell composition that is frequently studied across populations and disease conditions as a surrogate marker of systemic inflammation. The NLR ratio positively correlates with advancing age and with the degree of obesity, especially in the context of metabolic syndromes and type 2 diabetes. As such, the NLR ratio is indicative of low-grade inflammation, ‘inflammaging’ and obesity-associated inflammation, and is a poor prognostic factor in COVID-19. This observation indicates that individuals with such underlying

immune-system conditions either fail to develop productive antiviral immune responses or are more prone to develop uncontrolled, exuberant responses upon infection, leading to hyperinflammation and acute respiratory distress syndrome, characteristic of severe COVID-19. Older individuals typically produce weaker type I IFN responses upon viral infection, which further worsen the situation. Also, additional markers of inflammaging and obesity-associated inflammation have been shown to be predictive of a severe COVID-19 course, such as NLRP3 activation, IL-6, IL-12 and IL-1β secretion and danger-associated molecular patterns, including high mobility group box 1 (HMGB1).

Immunodeficiencies

Since the beginning of the pandemic, there have been grave concerns over the risk of developing severe COVID-19 for individuals with immunodeficiencies or those taking immunosuppressive therapies. One systematic review found no statistically significant increased risk of severe COVID-19 in immunosuppressed patients, but other studies have shown an increased risk for patients with solid-organ transplants and some patients with cancer. Patients with cancer treated with checkpoint inhibitors are at particularly high risk of severe COVID-19, according to another recent report. It is important to note that type and degree of immunosuppression likely varies substantially among heterogeneous patient groups, and more detailed subset analyses are needed. This is also highlighted by an Italian study of patients with different forms of primary antibody deficiencies, in which patients with combined variable immunodeficiency, often associated with low-grade inflammation, developed severe COVID-19, while patients with similarly low antibody levels due to other forms of inborn errors of immunity generally experienced milder course of disease.

Complications of the Immune System

The chain of immunological events associated with SARS-CoV-2 is characterized by the evolution of adaptive immunity (mediated by T and B lymphocytes) to the virus. Guillain-Barré Syndrome (GBS) has been associated to COVID-19. This disease is

characterized by an abrupt evolution, with an inflammatory cascade of peripheral nerves and loss of the myelin sheath (polyneuropathy). GBS was reported in clinical studies of adult, young, and child patients during or after coronavirus infection. Symptoms ranged from severe respiratory complications to motor paralysis. These symptoms have been associated—by different authors—to the physiological stimulation of inflammatory identified 456 rheumatic patients with a mean age of 63 years, and demonstrated the highest risk factor for severe COVID-19 (28.1%) in positive patients in whom immunosuppressants were used continually. These results corroborated studies by Haberman et al. Addi- tionally, a cross-sectional study of the impact of COVID-19 on rheumatic patients [observed that those affected exhibited arthralgia, myalgia, and weakness, with manifesta- tions that preceded COVID-19 respiratory symptoms.

Autoinflammatory conditions were reported in children, including Kawasaki disease (KD). This disease predominantly affects children under five years old and is character- ized as an acute inflammatory process in small and medium caliber vessels, exhibiting more cardiac involvement and a greater inflammatory response with macrophage activa- tion. Furthermore, myocarditis was found in young patients without any previous cardiac morbidity, with patients classified as critical, and with high cytokine secretion, showing acute respiratory distress syndrome. Despite the significant increase in KD cases after the start of the pandemic, further studies are needed to prove clinical association.

Molecular Mechanisms of Immune System Complications

A specific cytokine profile is associated with several factors in the severe stage of this disease, including: induction of interferon production, interleukin (ILs) 2 and 7 secretion, and the stimulation of granulocyte activation and production of tumor necrosis factor (TNF), causing intravascular hyperinflammation with changes in angiogenesis and coagulation. In addition to the understanding of the immune response to COVID-19, the association of symptoms with autoimmune diseases suggests that SARS-CoV-2 may trigger secondary diseases associated with a temporary immunosuppression profile and the presence of the virus.

3.2. Complications of the Hematological System

The pathophysiology of infection caused by COVID-19 involves several essential organic systems for maintaining homeostasis. The direct effect of SARS-CoV-2 hyper- inflammation induces the production of endogenous chemical substances that promote the alteration of vascular hemostasis. Blood coagulation is directly affected by the release of procoagulant and proinflammatory cytokines which activate disseminated intravascular coagulation and the formation of thromboembolic states that can aggressively affect various tissues, especially those that are more sensitive to ischemic processes, such as pulmonary, cardiovascular, and cerebrovascular tissues.

Molecular Mechanisms of Hematological System Complications

The SARS-Cov-2 cell entry mechanism is mediated by the hACE2-R receptor, which is expressed in several tissues, (e.g., lung, heart, intestinal smooth muscle, liver, and kid- neys) as well as in immune cells and the vascular endothelium. When the virus binds to the hACE2-R, it is sequentially internalized, leading to a decreased density of the receptor on the vascular tissue. This is associated with negative regulation of hACE2-R activity and accumulation of angiotensin II (Ang II), which causes vasoconstriction, profi- brotic, and proinflammatory effects, as well as inflammation and tissue fibrosis. The increased stimulation of inflammatory cytokines IL-1 and IL-6 by the activated M1 phe- notype macrophages (Interferon-7) and the excessive activity of Ang II bring endothelial activation, increased permeability, and coexpression of adhesion molecules, thus gener- ating a prothrombotic phenotype. Furthermore, this is verifiable via an increased production of other substances (e.g, plasminogen activator inhibitor factor I (PAI), tissue factor (TF), and von Willebrand factor (vWF)), which generate hemostatic changes that leave the endothelium inflamed, preadhesive,

and prothrombotic. This denotes ongoing tis- sue damage, which causes endotheliitis, mediated by SARS-CoV-2 directly invading endothelial cells.

The SARS-CoV-2 pathophysiology is complex, affecting several organs and systems; however, the cardiopulmonary system is severely affected.

Dysfunctions involving the respiratory system are among the most aggressive events associated with exacerbated immune responses caused by viral infection. The cytokine storm activates defense processes, stimulating biochemical pathways and leading to the production of tissue injury markers and the collapse of lung tissue. Among the main associated pathologies, we can highlight: respiratory failure, pulmonary thromboembolism, pulmonary embolism, pneumonia, pulmonary vascular damage, and postviral pulmonary fibrosis. Pulmonary vein throm- bosis is rarely found, but can present together with dyspnea, cough, chest pain, and/or hemoptysis, causing systemic arterial embolism. Systemic arterial embolism, in turn, is associated with venous and arterial thromboembolism pathogenesis in hypercoagulable states in patients with COVID-19. In a study, clinical and pathological findings indicated the presence of diffuse bilateral alveolar damage (DAD) in all the patients with SARS-CoV-2. Macroscopic and microscopic necropsy findings in patients who had severe COVID-19 infection demonstrated the presence of a major pulmonary pathology, described as a common complication, in patients with COVID-19. The complication over- lapped with acute bronchopneumonia (secondary), which was present in 78.6% of the patients and could be considered the main cause of death. Another important finding, in addition to that main pathology, was the presence of thrombotic/thromboembolic vascular occlusions. These hematological changes have been classified into five types of pulmonary thrombi: (I) capillary microthrombi; (II) thrombi partially organized in medium- sized pulmonary arteries with complete occlusion of the vessel; (III) thrombi not organized in medium-sized pulmonary arteries that do not completely fill the lumen of the vessel and probably represent thromboembolism instead of thrombosis; (IV) bone marrow emboli; and (V) septic pulmonary thromboemboli.

Pulmonary thrombi in medium-sized arteries were observed in 35.7% of patients, causing pulmonary infarction and/or pulmonary hemorrhage. After the autopsy results of the patients with postviral infection, it was confirmed that COVID-19 is a disease with a systemic characteristic. Because of a high involvement of the lungs in the infection, it increases the risk of cardiac and vascular complications, including acute myocardial injury and thrombotic/thromboembolic events that can affect other organs. Despite the evidence of pathological changes to secondary acute bronchopneumonia, it has been described as one of the most common complications in patients with COVID-19 and may be the leading cause of death.

Study questions.

1. Epidemiology of SARS-CoV-2 virus infection
2. The structure of the SARS-CoV-2 virus
3. Pathogenesis of COVID-19
4. Ways of SARS-CoV-2 infection and anti-epidemic quarantine measures
5. Symptoms of COVID-19
6. Features of COVID-19.
7. Diagnosis of SARS-CoV-2 and COVID-19.
8. Is there a specific etiotropic therapy for coronavirus infection?
9. Types of vaccines against SARS-CoV-2

1. **Control questions**
2. VIROLOGY AND TRANSMISSION
3. How is SARS-CoV-2 (the virus that causes COVID-19) transmitted?
4. What is the incubation period for COVID-19?
5. What are some of the important SARS-CoV-2 variants?
6. CLINICAL PRESENTATION
7. What are the clinical presentation and natural history of COVID-19?
8. What factors are associated with severe COVID-19?
9. COMPLICATIONS AND ASSOCIATED SYNDROMES
10. What are the major cardiac complications in patients with COVID-19? And how often do they occur?
11. What are the major thrombotic complications in patients with COVID-19?
12. What are the most common dermatologic syndromes associated with COVID-19?
13. What is multisystem inflammatory syndrome associated with COVID-19?
14. What is "long-COVID"?
15. CLINICAL EVALUATION
16. Is there a way to distinguish COVID-19 clinically from other respiratory illnesses, particularly influenza?
17. When should patients with confirmed or suspected COVID-19 be advised to stay at home? Have an in-person clinical evaluation?
18. LABORATORY EVALUATION
19. What laboratory abnormalities are commonly seen in patients with COVID-19?
20. What are the major coagulation abnormalities in patients with COVID-19?
21. DIAGNOSTIC TESTING
22. What are the different types of tests for COVID-19?
23. How accurate is RT-PCR for SARS-CoV-2? Should two tests be performed or one?
24. What are the indications for testing asymptomatic individuals?
25. When is the best time to test for COVID-19 following an exposure?
26. Can SARS-CoV-2 variants be reliably detected by available diagnostic assays?

Practical skills:

HOME CARE

1. Are there any COVID-19-specific therapies available for non-hospitalized patients?
2. What advice should be given to patients with known or presumed COVID-19 managed at home?
   a. How long should patients cared for at home stay isolated?
   b. What is the significance of a persistently positive RT-PCR for weeks after illness?
3. HOSPITAL CARE
   a. What is the preferred approach to oxygenation?
   b. When are antiviral treatment, glucocorticoids, and other COVID-19-specific therapies indicated? And which agents are preferred?
   c. Is anticoagulation indicated in all hospitalized patients? And if so, how much?
4. OTHER MEDICATION CONSIDERATIONS
   a. Should I use acetaminophen or NSAIDs when providing supportive care?
   b. Do ACE inhibitors and ARBs increase the likelihood of severe COVID-19?
5. SPECIAL POPULATIONS
   a. Asthma/COPD
      i. Should patients using inhaled glucocorticoids for asthma or COPD be advised to stop these medications to prevent COVID-19?
      ii. Should patients with COVID-19 and an acute exacerbation of asthma or COPD be treated with systemic glucocorticoids?
   b. Pregnancy, delivery, and breastfeeding
      i. What special considerations are there for pregnant and breastfeeding women?
   c. Pediatrics
      i. What special considerations are there for children?
   d. Other special populations
      i. What considerations are there for other special populations?
6. PREVENTION AND INFECTION CONTROL
   a. Are any medications available to prevent COVID-19 following exposure?
   b. What PPE is recommended for health care workers taking care of patients with suspected or confirmed COVID-19?
   c. What type of room should patients with known or suspected COVID-19 be placed in?
   d. Should individuals who are fully vaccinated continue to wear masks and physically distance?
7. VACCINATION AND IMMUNITY
   a. Immunity and vaccine efficacy
      i. Does protective immunity develop after SARS-CoV-2 infection? Can reinfection occur?
      ii. How efficacious is vaccination at preventing symptomatic COVID-19?
      iii. How effective is vaccination against the Delta variant?
      iv. Does vaccine efficacy wane over time?
      v. Does vaccination prevent asymptomatic transmission?
      vi. Have breakthrough infections been reported following vaccination?
   b. Vaccine availability and indications for vaccination
      i. Which vaccines are currently available in the United States? Worldwide?
      ii. What are the indications and contraindications to vaccination?
      iii. Who is eligible for a third dose of an mRNA vaccine? And when should it be administered?

c. Adverse effects

i. What adverse effects are associated with vaccination?

ii. Are COVID-19 vaccines associated with thrombotic complications? If so, which vaccines?

iii. Can analgesics or antipyretics be taken for side effects following vaccination?

d. Vaccine administration

i. Can other vaccines be given with COVID-19 vaccine?

ii. What if the second dose of an mRNA vaccine cannot be given because of a prior reaction?

iii. Should people who have had SARS-CoV-2 infection be vaccinated? If so, when? What if a patient acquires COVID-19 after the first dose?

iv. When administering a third dose of an mRNA vaccine to eligible individuals, should the same vaccine type as the initial two doses be used?

8.