

COVID-19

UNCOVERED

PATHOLOGY, ETIOLOGY, PATHOGENESIS, TRANSMISSION, OUTCOMES
AND LAB MEDICINE BEHIND THE COVID-19 PANDEMIC





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by Dr. M, Dr. R, Dr. S, Dr. A

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We/I acknowledge that Western University is located on the traditional territories of the Anishinaabek (Ah-nish-in-a-bek), Haudenosaunee (Ho-den-no-show-nee), Lūnaapéewak (Len-ahpay- wuk) and Chonnonton (Chun-ongk-ton) Nations, on lands connected with the London Township and Sombra Treaties of 1796 and the Dish with One Spoon Covenant Wampum. This land continues to be home to diverse Indigenous Peoples (First Nations, Métis and Inuit) whom we recognize as contemporary stewards of the land and vital contributors of our society.

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https://www.schulich.uwo.ca/PathOER/pulse/infectious_diseases/index.html

CONTENT

1

Introduction to COVID 19

2

Viral Entry & Mechanisms of Infection

3

Spread of Infection Through the Body and Immune Response

4

Disease Outcomes and Opportunistic Infections

5

Diagnostics, Lab Medicine & Therapies

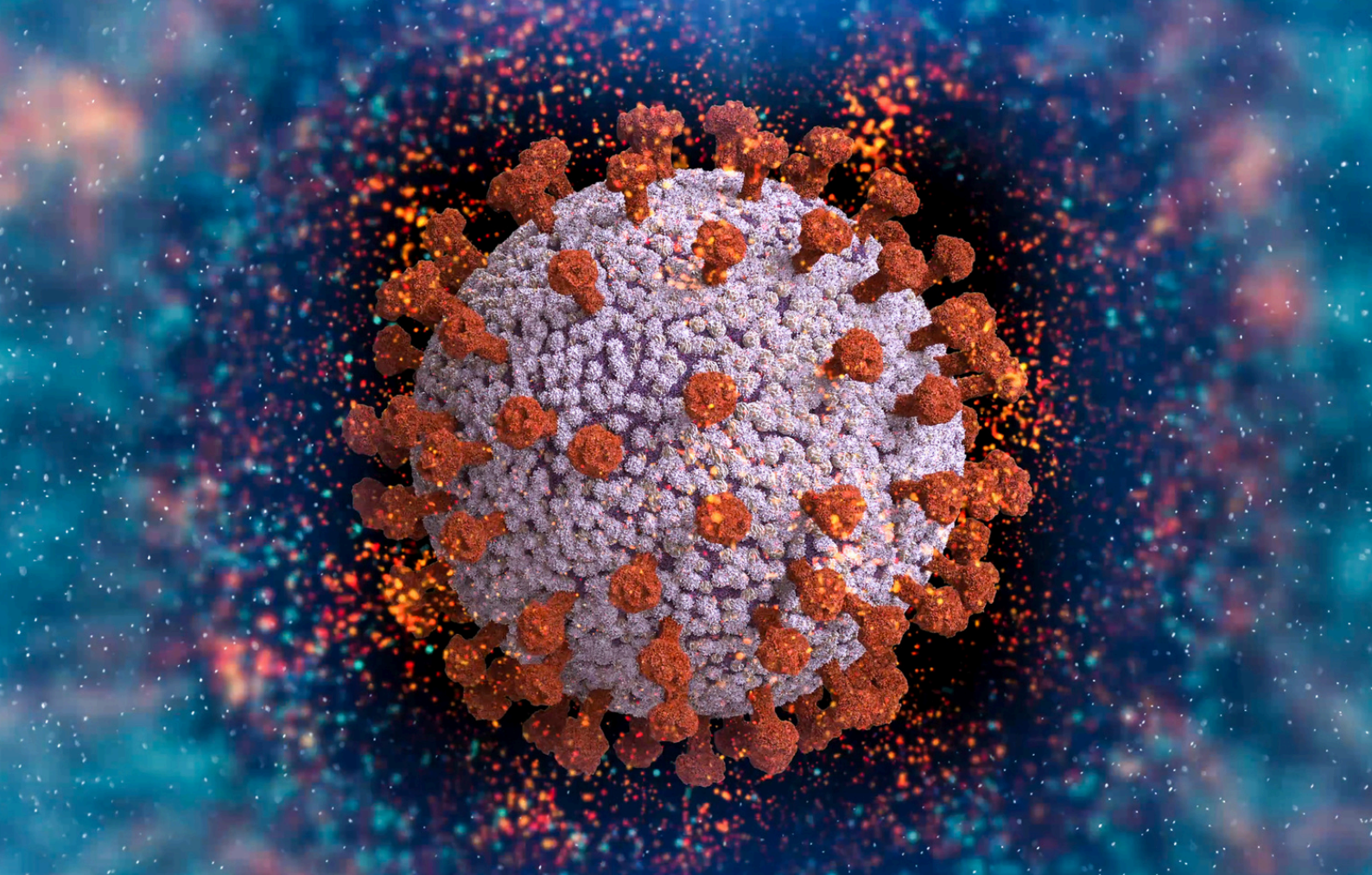
CHAPTER

1

COVID-19 OVERVIEW

DR. M





Chapter 1

INTRODUCTION

Diagnostic

COVID 19

Coronavirus disease 2019, or COVID-19, is the clinical disease caused by infection with severe acute respiratory syndrome coronavirus 2, SARS-CoV-2. Although it is often first understood as a respiratory infection, COVID-19 is more accurately viewed as a dynamic disease process whose severity depends on the interaction between viral structure, tissue tropism, host immune responses, and downstream inflammatory and vascular injury. In many individuals, infection remains limited to the upper respiratory tract and produces mild or moderate illness. In others, however, infection progresses into the lower respiratory tract, damages the alveoli, disrupts gas exchange, and contributes to severe complications including acute respiratory distress syndrome, thrombosis, and multiorgan dysfunction.¹

Diagnostic Multiple Choice Questions

1. Which statement best distinguishes SARS-CoV-2 from COVID-19?
A. SARS-CoV-2 is the immune response, while COVID-19 is the viral genome
B. SARS-CoV-2 is the virus, while COVID-19 is the clinical disease it causes
C. SARS-CoV-2 is the severe form, while COVID-19 is the mild form
D. SARS-CoV-2 refers only to pulmonary infection, while COVID-19 refers only to systemic infection
2. Which viral structure is most directly responsible for host cell recognition and entry?
A. Envelope protein
B. Membrane protein
C. Spike protein
D. Nucleocapsid protein
3. Which host receptor is the main entry receptor for SARS-CoV-2 discussed in your material?
A. DPP4
B. ACE2
C. CD4
D. CCR5
4. What is the main role of TMPRSS2 in SARS-CoV-2 infection?
A. It packages viral RNA
B. It activates the spike protein to facilitate entry
C. It produces interferon
D. It prevents membrane fusion
5. Which statement best describes the TMPRSS2 pathway compared with the endosomal pathway?
A. TMPRSS2 entry is slower and less efficient in lung epithelial cells
B. Endosomal entry is the dominant route in lung epithelial cells
C. TMPRSS2-mediated entry is relatively rapid, while endosomal entry is slower and broadens cell tropism
D. Both pathways are identical except for the receptor used
6. After successful early entry, what feature of the SARS-CoV-2 genome helps the virus begin replication quickly?
A. It is a DNA genome that must first enter the nucleus
B. It functions directly as messenger RNA
C. It cannot be translated until host DNA is degraded
D. It replicates only after antibody binding
7. In your chapter flow, severe COVID-19 is best understood as:
A. A disease determined entirely by viral load
B. A disease that stays confined to the upper airway

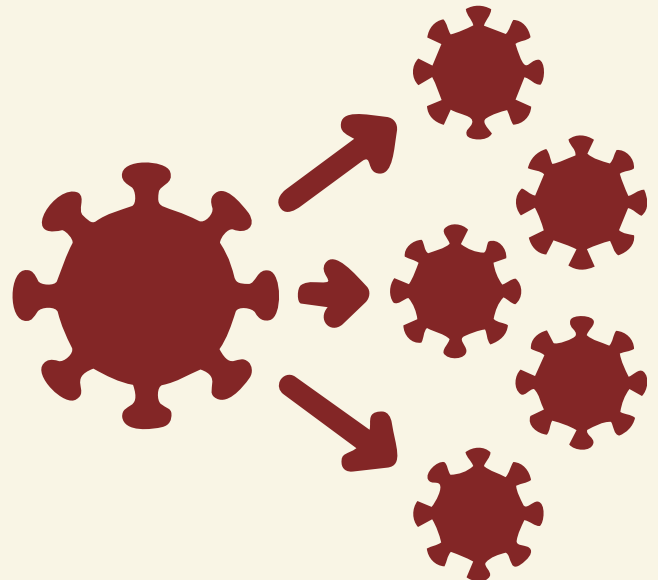


This textbook follows that progression step by step. Rather than presenting COVID-19 as a single static condition, the following chapters examine how the virus enters host cells, how infection spreads through the body, how immune and vascular responses shape disease severity, and how these biological processes inform diagnosis and treatment. This structure allows learners to connect molecular events with pathological change and, ultimately, with clinical outcomes.¹



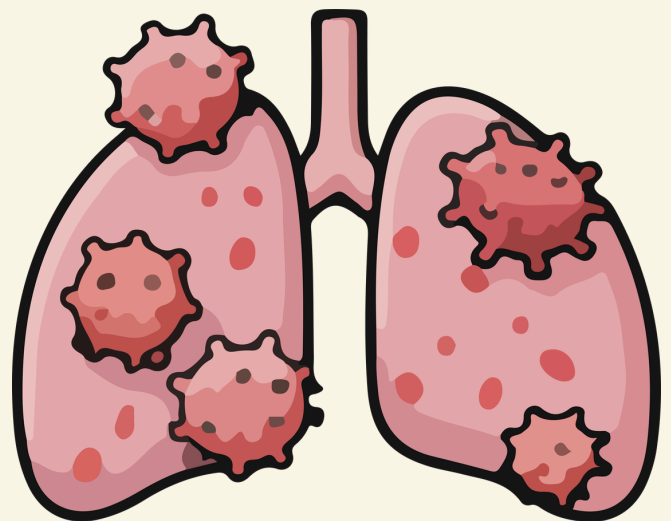
CHAPTER 2

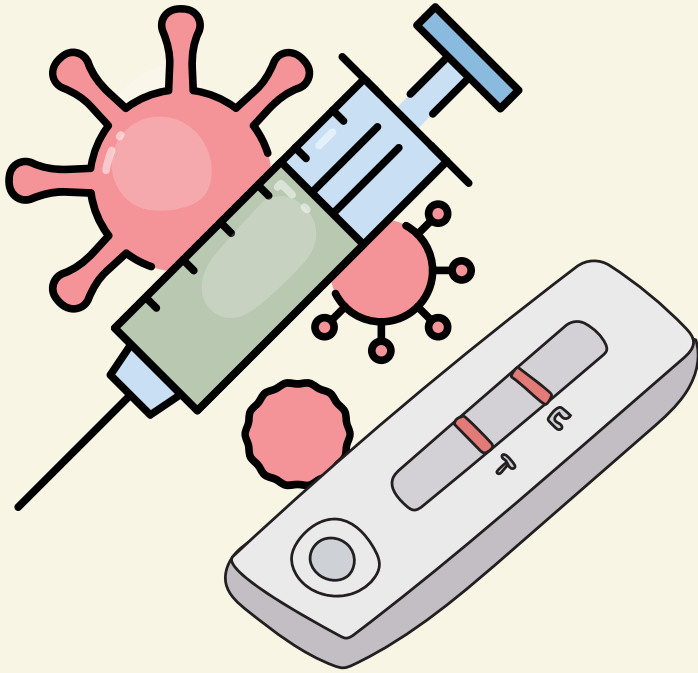
Chapter 2, Viral Entry and Mechanisms of Infection, focuses on the structure of SARS-CoV-2 and the molecular processes that permit host cell invasion. This chapter explains the major structural proteins of the virion, with particular attention to the spike protein and its interaction with ACE2. It also examines the role of host proteases such as furin and TMPRSS2, the conformational changes required for membrane fusion, and the early stages of viral replication once the viral genome enters the cytoplasm. Together, these mechanisms show how viral architecture directly shapes infectivity and tissue tropism.¹



CHAPTER 3

Chapter 3, Spread of Infection Through the Body and Immune Response, builds on viral entry by tracing what happens after infection is established. It addresses why SARS-CoV-2 can produce a wide spectrum of illness, from mild upper airway infection to severe pulmonary disease. This section follows the spread of infection from the nasopharynx to the alveoli and examines how innate immune sensing, type I and type III interferon responses, and adaptive immunity influence whether infection is contained or progresses. It also introduces the key idea that severe COVID-19 shifts over time from being largely virus driven to increasingly host response driven.¹





BIOLOGICAL AND SOCIAL DETERMINANTS OF SEVERE DISEASE

An introduction to COVID-19 should also acknowledge that disease severity and outcomes are not shaped by biology alone. As shown in the textbook factors you attached, severe COVID-19 is associated with age, comorbidities, socioeconomic background, socially defined race, gender, laboratory abnormalities, and certain genetic factors.² The screenshot is especially useful for framing this discussion because it shows that differences in severe disease burden are linked not only to clinical risk, but also to broader social and structural inequities rather than simple biological difference.²

CHAPTER 4

Chapter 4, Disease Outcomes and Opportunistic Infections, examines the pathological consequences of failed containment. It explains how COVID-19 can progress from pneumonia to diffuse alveolar damage, hypoxemia, acute respiratory distress syndrome, endothelial dysfunction, thrombosis, and broader systemic involvement. This chapter emphasizes that severe disease is not determined by viral load alone, but by the interaction among viral replication, immune dysregulation, and vascular injury.¹

CHAPTER 5

Chapter 5, Diagnostics, Lab Medicine, and Therapies, translates pathogenesis into clinical practice. This chapter reviews the major diagnostic strategies used to detect SARS-CoV-2 and explains how test interpretation depends on timing, viral load, specimen quality, and disease stage. It also surveys major therapeutic approaches, including antivirals, immunomodulators, anticoagulation, and supportive care, emphasizing that treatment effectiveness depends on whether disease is in an early viral phase or a later immune mediated phase.¹

For this reason, COVID-19 should be studied not only as a viral disease, but also as a disease whose burden is distributed unequally across populations. This perspective is important because it connects pathophysiology to the unequal social realities in which infection occurs, including differences in exposure risk, underlying health conditions, access to care, and the timing of diagnosis and treatment.² A complete understanding of COVID-19 therefore requires attention to both biological mechanisms and the broader structural conditions that shape who is most affected.²

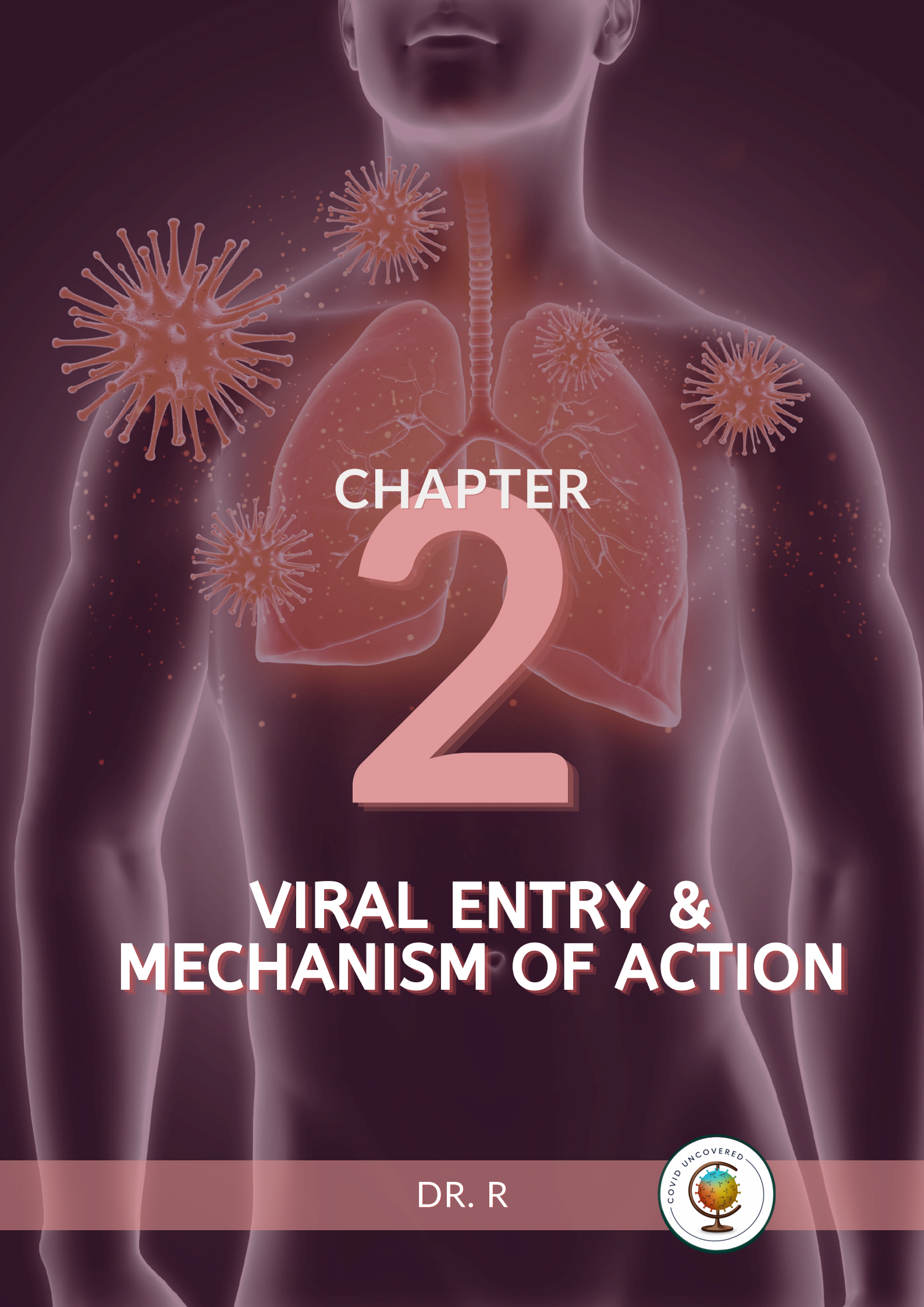


GUIDED LEARNING FLASHCARDS

At the end of each chapter, you can use this spreadsheet to generate flashcards to help with your learning.

COVID-19 Flashcard Workbook			
A formatted workbook for studying and exporting your chapter-based COVID-19 flashcards.			
Deck Summary			
Total cards	119	Code	
Unique chapters	5	Ch1	
Longest chapter	Viral Entry & Mechanisms of Infection	Ch2	
Shortest chapter	Introduction to COVID-19	Ch3	
Chapter Guide		Ch4	
Code	Chapter Title	Primary Focus	
Ch1	Introduction to COVID-19	Definitions, disease spectrum, risk factors, and the bridge from molecular pathogenesis to clinical disease.	
Ch2	Viral Entry &	Virion structure, ACE2 binding, furin/TMPRSS2	Di
Overview	Study Deck	Anki Import	Quizlet Import





CHAPTER

2

VIRAL ENTRY & MECHANISM OF ACTION

DR. R





Chapter 2

ENTRY AND MECHANISMS

Learning Goals

By the end of this section, learners should be able to:

1. Classify SARS-CoV-2 within the coronavirus family and describe the structural organization of the virion.
2. Identify the major structural proteins (S, M, E, and N) and explain their roles in viral structure and function.
3. Explain how the spike protein mediates host cell recognition through ACE2 binding and conformational changes in the RBD.
4. Describe how host proteases (e.g., furin and TMPRSS2) activate the spike protein and facilitate viral entry.
5. Compare the major pathways of viral entry and outline the key steps of membrane fusion leading to genome release.
6. Outline the early stages of viral replication, including translation of the viral genome and formation of the replication-transcription complex.

INTRODUCTION

COVID-19 is an RNA based virus belonging to the SARS - CoV2 family. Infection by COVID-19 is not governed by a single step, rather it depends on a series of processes including spike-protein mediated receptor binding, proteolytic activation, membrane fusion and viral replication.

In this section, we give a detailed explanation of the structure of the virus including all the proteins involved in host-cell recognition and infection followed by an account of the step by step process of infection, ultimately illustrating how underlying viral structure affects its ability to infect host cells.

SARS - COV2 FAMILY

SARS - CoV2 is a beta coronavirus belonging to the Orthocoronaviridae subfamily within the Coronaviridae family and Nidovirales order. It is widely believed to have originated in bats, with transmission to humans occurring either directly, or through intermediate hosts. Like other coronaviruses, SARS - CoV-2 is an enveloped virus with a positive-sense single stranded RNA genome, enabling it to rapidly initiate infection upon entry into host cells³.

The spike protein forms prominent trimeric projections on the viral surface and is responsible for host cell recognition and entry. The membrane protein provides structural integrity to the virion, while the envelope protein plays a role in viral assembly and can oligomerize to form ion channels. Some coronaviruses encode hemagglutinin esterase (HE), although this protein is not a major structural component of SARS-CoV-2. Enclosed within the viral envelope is the nucleocapsid (N) protein, which binds and packages the viral RNA genome.³

ARCHITECTURE OF THE CORONAVIRUS

The SARS-CoV-2 virion is spherical in shape, with a diameter of approximately 80–120 nm. Its lipid envelope contains three major structural proteins: the spike (S) protein, the membrane (M) protein, and the envelope (E) protein.

HOST CELL RECOGNITION & ENTRY

Viral entry into host cells is mediated by the spike (S) protein, a homotrimeric class I fusion protein composed of two functional subunits. The S1 subunit is responsible for receptor binding, while the S2 subunit mediates membrane fusion.

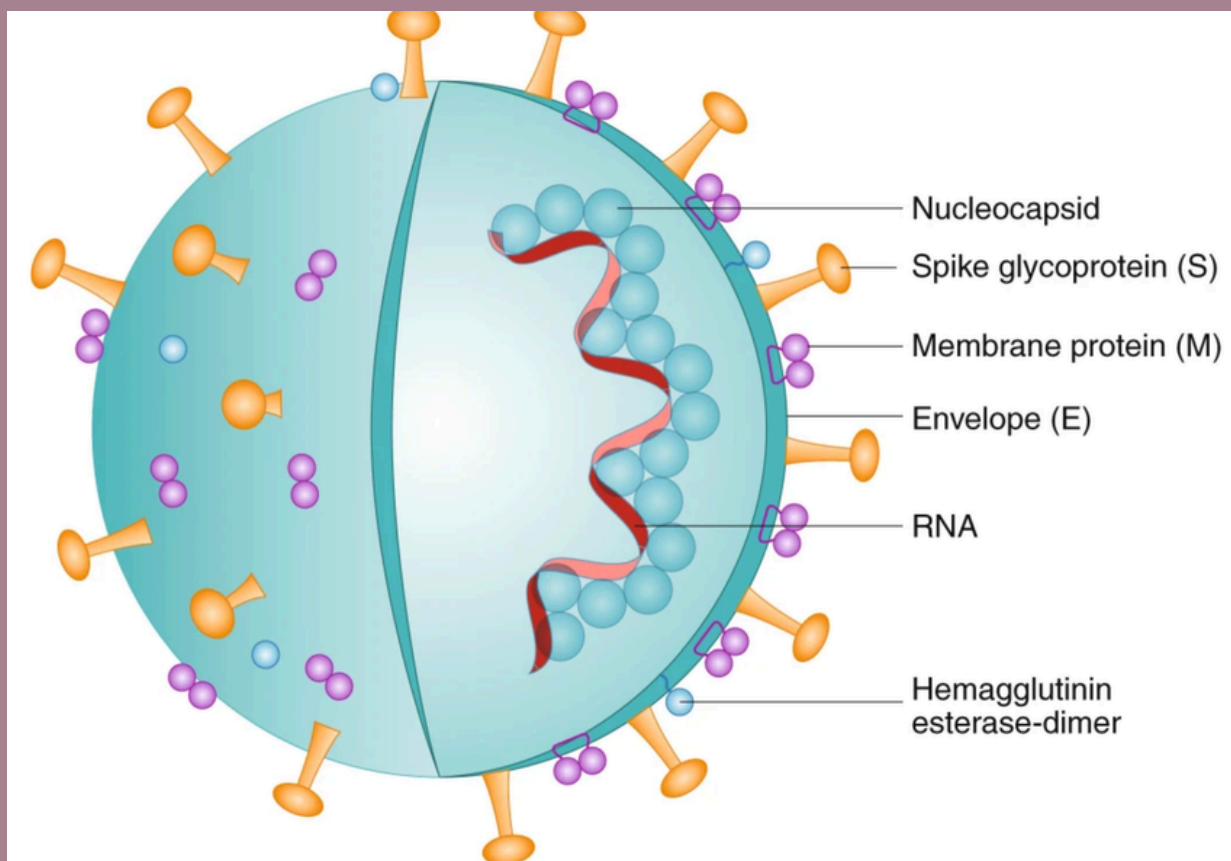


Figure 2.1. Detailed Labelled diagram of the Coronavirus virion, image was taken from Florindo et al, 2020.⁴

During viral assembly within an infected host cell, the spike protein undergoes cleavage at the S1/S2 boundary by host proteases such as furin. SARS-CoV-2 contains a unique polybasic furin cleavage site at this junction, which pre-activates the spike protein and enhances its ability to infect host cells by facilitating subsequent activation steps. The S1 subunit contains the receptor-binding domain (RBD), which interacts with the host cell receptor angiotensin-converting enzyme 2 (ACE2). Within the RBD lies the receptor-binding motif (RBM), a flexible loop that directly contacts ACE2. The RBM can adopt two conformations: an “up” conformation in which the RBM is exposed and capable of binding ACE2, and a “down” conformation in which the RBM is hidden and binding is not possible. Mutations within the RBM can significantly alter binding affinity and contribute to differences in viral transmissibility. Following receptor binding, the spike protein must undergo a second cleavage at the S2' site to become fully activated for membrane fusion. This step can occur through two distinct pathways.

In the TMPRSS2-dependent pathway, cleavage occurs at the plasma membrane via the host serine protease TMPRSS2, which is highly expressed in respiratory, gastrointestinal, and urogenital epithelial tissues. This pathway is relatively rapid and is thought to be the dominant route of entry in lung epithelial cells.

Alternatively, the virus can enter via endocytosis, where cleavage is mediated by cathepsin L within acidified endolysosomal compartments.

The endosomal pathway is slower but provides an additional route for viral entry, enhancing the virus’s ability to infect a range of cell types⁵.

MEMBRANE FUSION

Following proteolytic activation, the spike protein undergoes a series of conformational changes that drive membrane fusion. Upon ACE2 binding, the S1 subunit dissociates, allowing the S2 subunit to transition into an extended intermediate state. This exposes a hydrophobic fusion peptide, which inserts into the host cell membrane⁷.

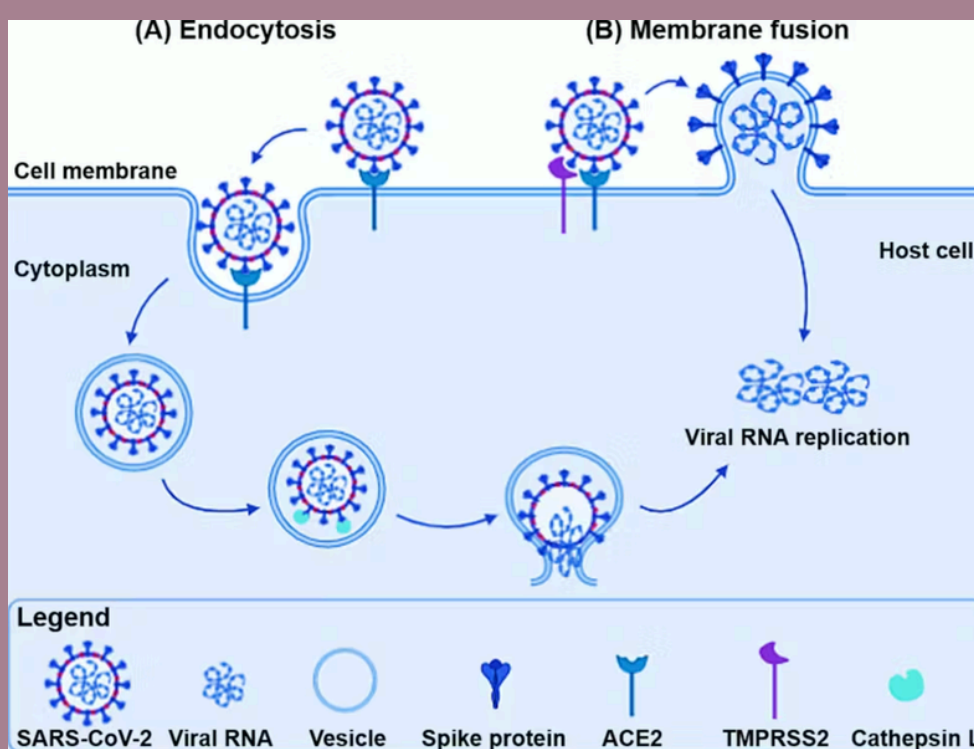


Figure 2.2. Entry pathways, of COVID-19 into the the cell via Endocytosis and Membrane Fusion, including cellular and viral components involved. Image was taken from Pišlar et al., 2020.⁶

The S2 subunit contains two heptad repeat regions, HR1 and HR2, which subsequently refold to form a stable six-helix bundle. This structural rearrangement brings the viral and host membranes into close proximity, facilitating their merger.⁷

EARLY VIRAL REPLICATION

Once released into the cytoplasm, the SARS-CoV-2 genome functions directly as messenger RNA due to its positive-sense polarity. Host ribosomes translate the viral RNA into two large polyproteins, PP1A and PP1AB, which are subsequently cleaved by viral proteases into a series of nonstructural proteins. These proteins assemble into the replication-transcription complex (RTC), which is responsible for viral RNA synthesis. A key component of this complex is the RNA-dependent RNA polymerase, which generates new genomic RNA as well as subgenomic RNA required for the production of structural proteins.⁸

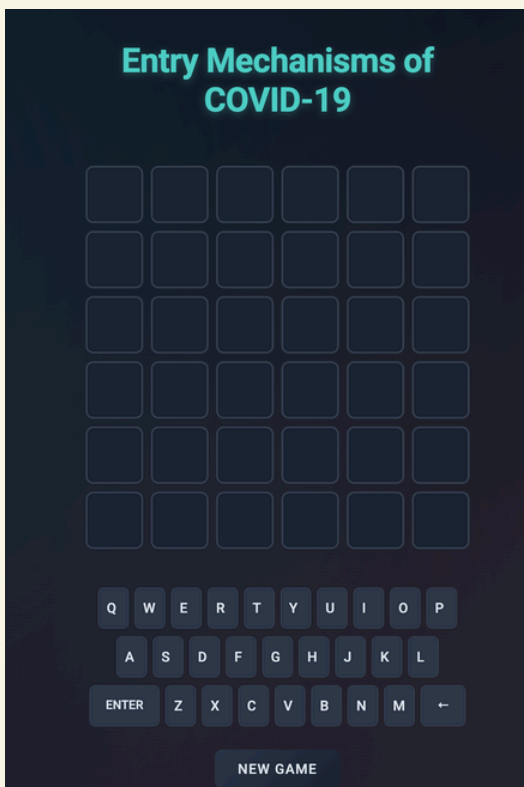
KEY TAKEAWAYS

- Entry via the TMPRSS2 pathway is rapid and primarily occurs in pulmonary epithelial cells.
- Entry via endocytosis is slower and enables infection of a broader range of cell types.
- Modifications to the spike (S) protein can alter host recognition and binding affinity.
- Both TMPRSS2- and endocytosis-mediated entry require the presence of ACE2 on host cells.
- The receptor-binding domain (RBD) of the spike protein must be in the “up” conformation to bind the ACE2 receptor.
- The presence of a furin cleavage site pre-activates the spike protein and enhances viral infectivity

TEST YOUR KNOWLEDGE

1. How does the structure of SARS-CoV-2 help it infect host cells quickly?
2. How do changes in the spike protein allow it to bind ACE2 and cause membrane fusion?
3. If the receptor-binding domain (RBD) is more often in the “up” position, how would this affect infectivity? Why?
4. What are the differences between the TMPRSS2 pathway and the endosomal pathway of viral entry? How do these affect which cells the virus can infect?
5. Why is the furin cleavage site important for SARS-CoV-2 entry into host cells?
6. What are the main steps that occur after ACE2 binding until the viral genome enters the cell?
7. How does the SARS-CoV-2 RNA genome allow it to be quickly translated and replicated inside the host cell?

GAME



ANSWERS

(Note answers need not be identical; provided below are model/sample answers)

1. SARS-CoV-2 is built in a way that makes infection very efficient. Its RNA genome can be used immediately by the host cell to make viral proteins, so there's no delay after entry. The spike proteins on its surface are specifically designed to recognize and attach to host cells. These spikes are also already partially activated, which helps the virus enter cells faster. Overall, its structure allows it to attach, enter, and start replicating very quickly.
2. The spike protein isn't rigid hence it can change shape depending on what stage of infection it's in. The part that binds to ACE2 can flip into an “up” position, making it easier to attach to the receptor. Once it binds, the spike is further activated by host enzymes. This triggers another shape change that exposes a fusion region. That region helps pull the viral and cell membranes together so they can fuse.
3. If the RBD is in the “up” position more often, it's easier for the virus to bind to ACE2. This increases the chances of successful infection. As a result, the virus can enter cells more efficiently and spread more easily. However, being more exposed might also make it easier for the immune system to detect. Still, overall, it would likely increase infectivity.
4. The TMPRSS2 pathway happens right at the cell surface, where a host enzyme activates the spike protein and allows immediate fusion. This is a fast and efficient way for the virus to enter cells, especially in the respiratory tract. The endosomal pathway is different because the virus is first taken into the cell and then activated inside endosomes. This process is slower but doesn't require TMPRSS2. Because of this, the virus can infect a wider variety of cells using the endosomal route.
5. The furin cleavage site allows the spike protein to be cut and activated before the virus even reaches a new cell. This gives the virus a head start during infection. Because the spike is already partially activated, it can enter cells more easily. It also means the virus doesn't rely as much on specific enzymes in the target cell. This helps it infect more types of cells and spread more efficiently.
6. After the virus binds to ACE2, the spike protein is further activated by host enzymes. This causes it to change shape and expose a fusion region. That region inserts into the host cell membrane and pulls the two membranes closer together. Eventually, the membranes fuse and form an opening. Through this opening, the viral RNA enters the cell.
7. The viral RNA acts like ready-to-use instructions for the host cell. As soon as it enters, the cell's ribosomes start making viral proteins. These proteins include enzymes needed to copy the viral genome. The virus quickly sets up its own replication system inside the cell. This allows it to produce many copies of itself in a short amount of time.

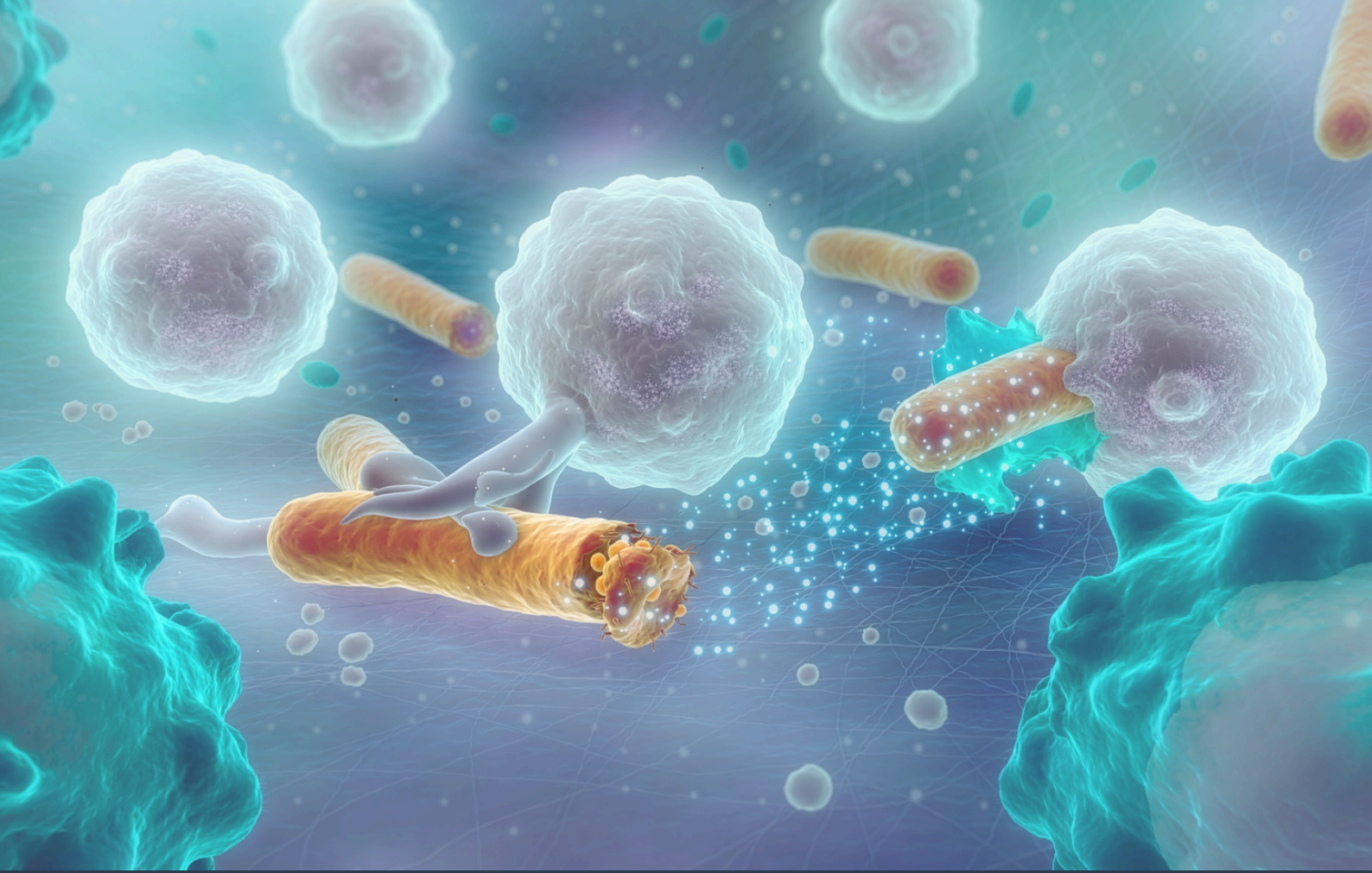
CHAPTER

3

SPREAD OF INFECTION THROUGH BODY AND IMMUNE RESPONSE

DR. M





Chapter 3

INFECTION SPREAD AND IMMUNE RESPONSE

Learning Goals

By the end of this section, learners should be able to:

1. Explain why COVID-19 presents as a spectrum of disease severity and identify host factors that influence progression.
2. Trace the spread of SARS-CoV-2 from the upper airway to the alveoli and explain how this changes the clinical significance of infection.
3. Describe how innate immune responses attempt early containment and how failed interferon responses increase severity.
4. Compare the roles of humoral and cellular adaptive immunity in viral control, recovery, and immune memory.
5. Distinguish between the relatively virus-driven early phase and the increasingly host-response-driven severe phase of COVID-19.
6. Integrate anatomical spread, immune dysregulation, endothelial injury, and thrombosis into one model of severe disease.

INTRODUCTION

The first thing to talk about is the difference between COVID-19 and SARS-CoV-2. COVID-19 is the clinical disease caused by SARS-CoV-2. One of its defining features is that it does not behave like a single uniform illness. In some individuals, infection remains mild and largely confined to the upper respiratory tract. In others, it progresses to viral pneumonia, acute respiratory distress syndrome, thrombosis, and multiorgan dysfunction.^{1-2,9-10}

This variation reflects host-pathogen interaction. The course of disease depends not only on the virus itself, but also on where infection begins, how well it is contained early, and whether later illness becomes dominated by dysregulated inflammation and vascular injury.^{1-2,9}

ETIOLOGIC AGENT AND HOST SUSCEPTIBILITY

SARS-CoV-2 is an enveloped positive-sense RNA coronavirus, but the virus alone does not determine the clinical course of disease. Severe outcomes are disproportionately concentrated among older adults and among individuals with comorbidities or multimorbidity, showing that host factors strongly influence whether infection remains localized or progresses to systemic disease.^{2,10} Figure 3.1a illustrates that predicted in-hospital mortality rises across age groups and increases further as the number of comorbidities increases, indicating that multimorbidity adds to age-related risk.¹⁰ Figure 3.1b complements this pattern by showing that comorbidities may contribute to severity through several overlapping mechanisms, including limited functional reserve, excessive inflammation, compromised lung function, and defective viral control.¹⁰

A useful way to understand this variation is through the balance between early viral control and inflammatory escalation. When early host defenses are effective, infection is more likely to remain localized and mild. When early control is impaired, viral burden can increase and create conditions that favour progression to severe pulmonary and systemic disease.⁹

Impaired type I interferon biology, including neutralizing autoantibodies against type I interferons, provides one example of failed early containment and helps explain why some patients are more susceptible to severe COVID-19.^{2,9,11,15}

Factors Linked To More Severe COVID-19 Cases

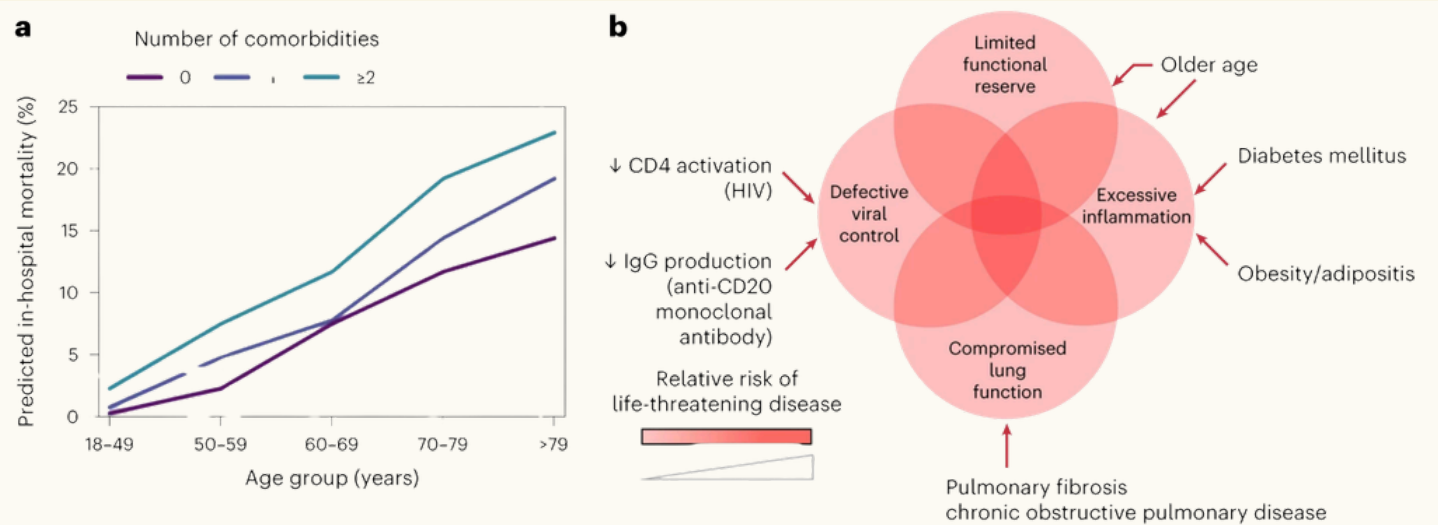
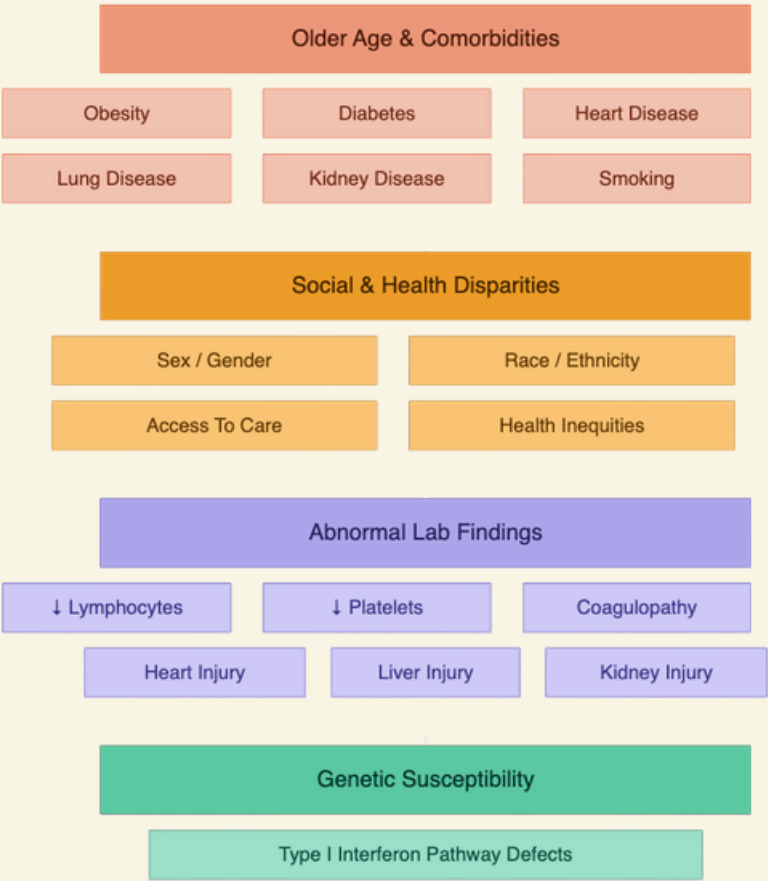


Figure 3.1. (a) Predicted in-hospital mortality across age groups by number of comorbidities, showing that mortality risk increases with both age and comorbidity burden. (b) Proposed overlapping mechanisms by which age and comorbidities may contribute to life-threatening COVID-19, including limited functional reserve, excessive inflammation, compromised lung function, and defective viral control. Taken from Russell et al.¹⁰

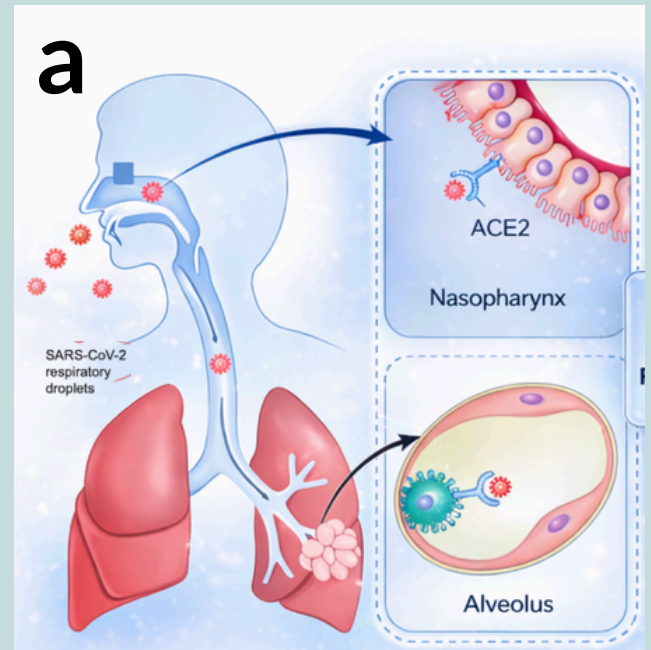
SPREAD OF INFECTION THROUGH THE BODY

UPPER AIRWAY ESTABLISHMENT AND CONTAGIOUSNESS

SARS-CoV-2 commonly establishes infection in the upper respiratory tract, especially the nasopharyngeal epithelium. As shown in Figure 3.2a, infection begins in the airway, where the virus can bind and replicate within respiratory epithelial cells.¹⁻² Viral replication can be high around the time of symptom onset, which helps explain why transmission can occur efficiently even when symptoms are still mild or nonspecific.^{1,12} When early antiviral control is effective, infection may remain mild or asymptomatic and not spread farther into the respiratory tract.^{2,9}

EXTENSION TO THE LOWER RESPIRATORY TRACT

In a subset of patients, infection extends from the upper airway into the lower respiratory tract and distal airspaces. Figure 3.2a also illustrates this progression into the alveoli, where the clinical significance of disease changes. Once alveoli become involved, the disease is no longer only a transmissible mucosal infection. It becomes a pulmonary disease with major consequences for oxygen exchange and respiratory function.¹⁻² Viral pneumonia, worsening hypoxemia, and progressive lung injury become more likely as alveolar involvement increases.¹⁻²



TRANSITION TO BROADER DISEASE

In severe COVID-19, the illness cannot be understood as merely more virus in more places. Instead, localized respiratory infection begins to produce body-wide consequences as inflammatory mediators enter the circulation, endothelial function becomes disrupted, and coagulation pathways are engaged.^{1-2,9,14-15} As summarized in Figure 3.2b, effective early control is associated with lower viral burden and milder disease, whereas suboptimal control can lead to higher viral load, systemic inflammatory responses, and severe pulmonary and multiorgan disease. Thus, although the anatomic route of infection begins in the airway, severe disease increasingly reflects vascular and immunologic involvement beyond the lungs.^{1-2,9,14-15}

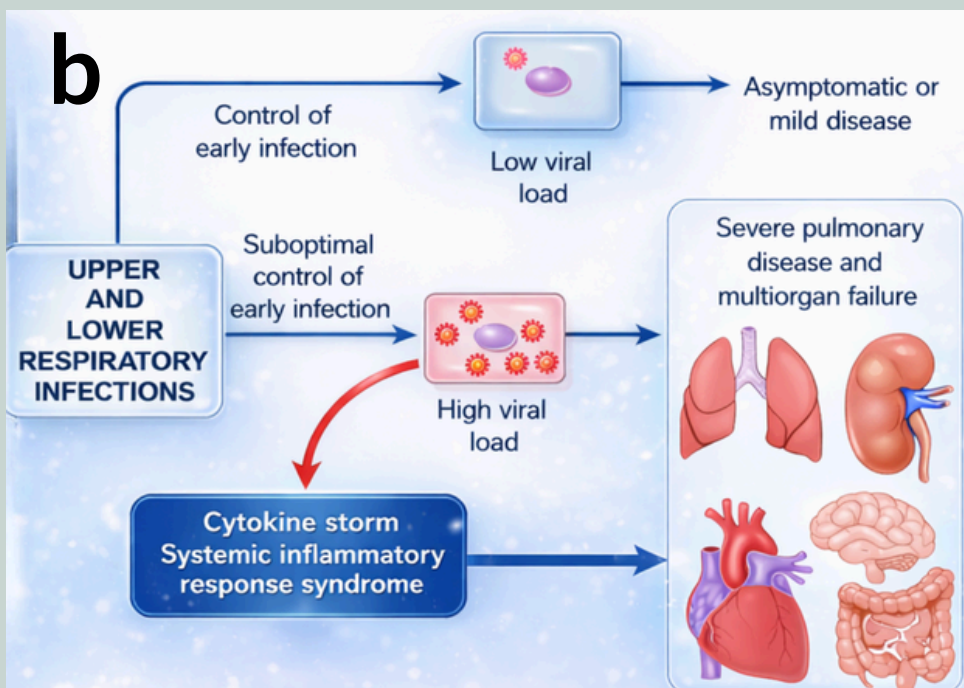


Figure 3.2. (a) Schematic of SARS-CoV-2 infection involving the upper and lower respiratory tract, illustrating viral establishment in the nasopharynx and extension into the alveoli. (b) Simplified model showing how effective early control is associated with low viral load and mild disease, whereas suboptimal control can lead to high viral load, systemic inflammatory responses, and severe pulmonary and multiorgan disease. Adapted using ChatGPT from Robbins & Kumar basic pathology.²

IMMUNE RESPONSES TO SARS-COV-2

THE INNATE IMMUNE RESPONSE

Whether infection remains localized or progresses toward severe disease depends heavily on the innate immune response, the body's first line of defense. Airway epithelial cells and other innate immune cells detect viral components through pattern-recognition receptors and begin antiviral signaling. A major part of this early defense is the production of type I and type III interferons, which activate interferon-stimulated genes and help establish an antiviral state in surrounding tissues.^{2,9,13}

As shown in Figure 3.3, the timing and strength of the interferon response strongly influence disease progression. When type I and type III interferon responses are rapid and effective, viral expansion is more likely to remain limited, helping to keep infection localized and clinically mild.^{2,9,13} By contrast, when these responses are delayed, weakened, or neutralized, the virus gains an early advantage. Viral burden can rise, inflammatory signalling intensifies, and the risk of severe downstream disease increases.^{9,11,13}

This helps explain why severe COVID-19 is not simply a failure of innate immunity, but also a problem of innate immune dysregulation. As also reflected in Figure 3.3, poor interferon control is associated with increased viral replication and a greater likelihood of inflammatory complications. Severe disease is associated with increased cytokine production, including interleukin-6 and tumor necrosis factor, together with activated myeloid-lineage responses that intensify tissue injury, endothelial dysfunction, and coagulopathy.^{2,9,11,13-15}

ADAPTIVE IMMUNE RESPONSE AND IMMUNE MEMORY

As infection continues, adaptive immunity becomes increasingly important. Unlike innate immunity, which acts quickly but broadly, adaptive immunity is more targeted and develops over time. It includes both humoral immunity, which is mediated by antibodies, and cellular immunity, which is mediated by T cells.⁹

Humoral immunity includes both mucosal and systemic compartments. In the respiratory tract, mucosal IgA can help reduce viral establishment at the site of entry, while systemic neutralizing antibodies help limit viral spread and are most effective before or early during the virus-driven phase of illness.^{9,16} These antibody responses help support early containment and reduce the chance of progression.

Cellular immunity is also essential. SARS-CoV-2-specific CD4 and CD8 T-cell responses help control infection, limit disease severity, and support recovery.^{2,9,17} These responses can persist for months after primary infection, showing that adaptive immunity contributes not only to recovery from acute illness but also to longer-term immune memory.¹⁷

However, severe COVID-19 is commonly associated with lymphopenia, or reduced numbers of circulating lymphocytes, which is often treated as a marker of severe systemic illness and reduced immune competence.^{2,17,18} This means that adaptive responses may still be present, but they occur in the setting of broader immune disruption.

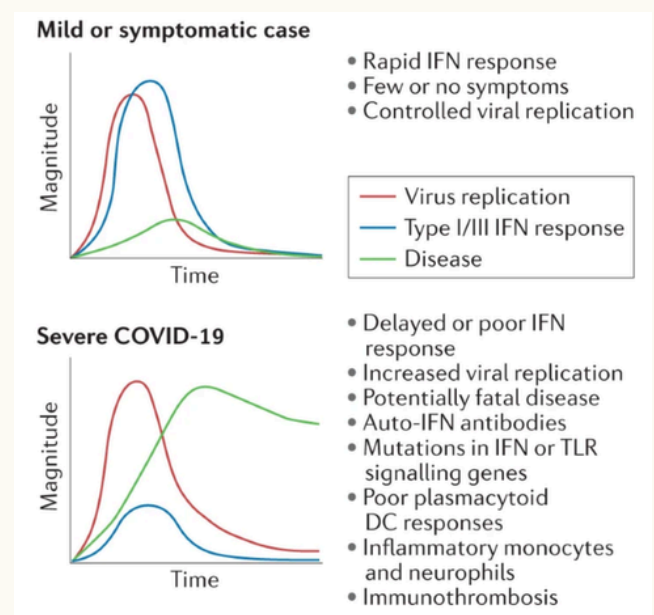


Figure 3.3. Rapid type I and type III interferon responses are associated with controlled viral replication and milder disease, whereas delayed or poor interferon responses are associated with increased viral replication and more severe disease.

Taken from Lamers and Haagmans.¹

BRINGING IT TOGETHER: DISEASE PROGRESSION OVER TIME

The course of COVID-19 can be understood as a shift in dominant disease drivers. Early infection is relatively virus-driven: SARS-CoV-2 seeds the upper airway, replicates locally, and is either contained or allowed to spread depending on the effectiveness of early innate defenses, especially interferon-linked antiviral programs.^{2,9,11-13} At this stage, transmission and viral burden are major concerns.

If early containment is incomplete, infection may extend into the alveoli, where pulmonary injury becomes more clinically important. From there, progressive and severe disease becomes increasingly host-response driven.^{2,9,14,15} Inflammatory amplification, endothelial dysfunction, and coagulation abnormalities begin to matter more than viral replication alone. This is why clinical deterioration in severe COVID-19 often aligns with escalating inflammation and vascular pathology rather than simply with continuously rising viral load.

Over approximately the first one to two weeks, adaptive immune responses expand, including virus-specific T-cell responses and rising neutralizing antibody activity.^{9,16,17} In many patients, this is followed by resolution and immune memory. In a subset, however, persistent immune activation or dysregulation may continue after acute infection.^{9,17,19}

Key Takeaways

- COVID-19 severity depends on host response.

The same virus can cause mild upper-airway infection or severe systemic disease.

- Infection usually starts in the upper airway.

High viral replication near symptom onset helps drive transmission.

- Spread to the alveoli worsens disease.

Lower-airway involvement is linked to pneumonia, hypoxemia, and lung injury.

- Early interferon responses matter.

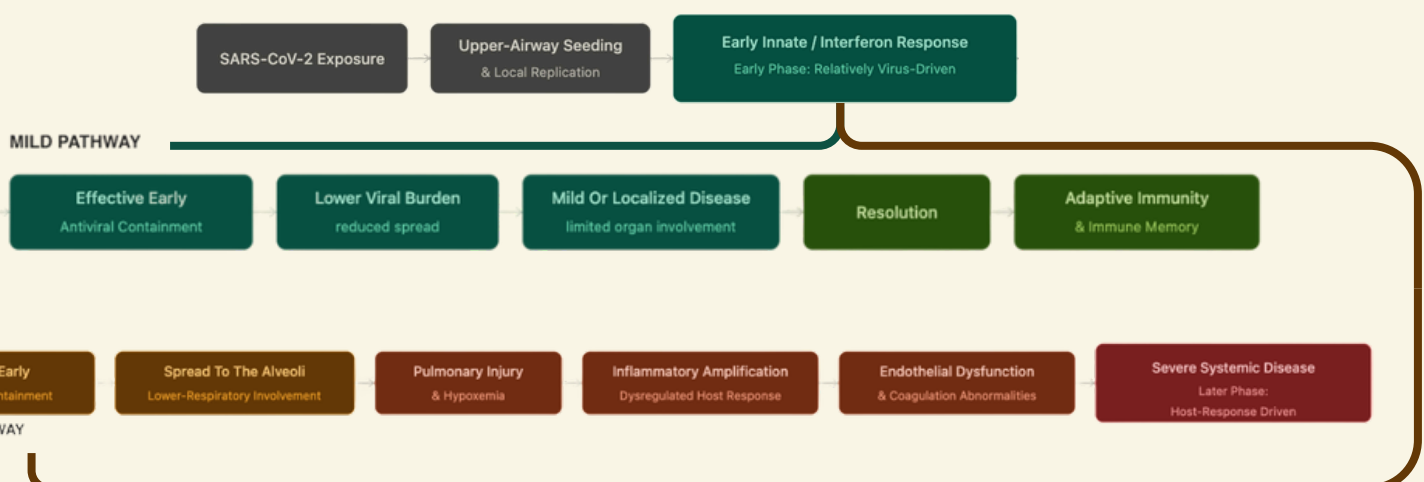
Effective early antiviral control helps keep infection localized; failed control increases severity.

- Adaptive immunity supports recovery.

Antibodies and T cells help limit infection and contribute to immune memory.

- Severe COVID-19 becomes increasingly host-response driven.

Inflammation, endothelial injury, and thrombosis become central to systemic disease.



TEST YOUR KNOWLEDGE

1. Why does COVID-19 range from mild upper-respiratory infection to severe pulmonary and systemic disease, and how do age, comorbidity, and impaired type I interferon biology help explain that variation?
2. Trace the spread of SARS-CoV-2 from the nasopharyngeal epithelium to the lower respiratory tract and alveoli. At what point does the disease shift from a mainly transmissible mucosal infection to a pulmonary disease with major consequences for oxygen exchange?
3. Why is upper-airway viral replication near symptom onset especially important for contagiousness, whereas alveolar involvement is more closely associated with viral pneumonia, hypoxemia, and respiratory failure?
4. How do type I and type III interferons help contain SARS-CoV-2 early in infection, and how can delayed, weakened, or neutralized interferon responses increase disease severity?
5. How do mucosal IgA, neutralizing antibodies, CD4 T cells, and CD8 T cells each contribute to viral control, recovery, and immune memory in COVID-19?
6. Why can severe COVID-19 show both adaptive immune responses and lymphopenia at the same time?
7. What does it mean to say that early COVID-19 is relatively virus-driven, whereas severe COVID-19 becomes increasingly host-response driven?
8. Starting with upper-airway infection, explain how failed early containment can lead to inflammatory escalation, endothelial dysfunction, immunothrombosis, and systemic complications.

GAME

BioStrands

COVID-19 Pathogenesis Edition · by Yashmi

LEARNING OBJECTIVE

Trace how SARS-CoV-2 spreads from initial entry to severe disease — connecting anatomical spread, failed antiviral control, and inflammatory escalation as interlinked mechanisms.

3 BOARDS — COMPLETE IN ORDER

1 From Entry to Severe Disease

Trace how SARS-CoV-2 spreads from the upper airway into the lungs and beyond.

2 Early Control vs Severe Disease

See how innate and adaptive immunity attempt to limit viral spread, and what happens when they fail.

3 When Inflammation Escalates

Discover how runaway cytokine signalling injures blood vessels and



ANSWERS

(Note answers need not be identical; provided below are model/sample answers)

1. COVID-19 ranges from mild to severe because severity depends on host susceptibility, not just the virus. Older age and comorbidities increase risk, and impaired type I interferon biology weakens early antiviral containment, allowing progression to severe pulmonary and systemic disease.
2. SARS-CoV-2 usually begins in the nasopharyngeal epithelium, then may spread into the lower respiratory tract and alveoli. The disease shifts from a mainly transmissible mucosal infection to a pulmonary disease once the alveoli are involved, because gas exchange becomes impaired.
3. Upper-airway viral replication drives contagiousness because viral load can be high near symptom onset. Alveolar involvement is linked to viral pneumonia, hypoxemia, and respiratory failure because it damages the part of the lung responsible for oxygen exchange.
4. Type I and type III interferons help contain SARS-CoV-2 early by inducing an antiviral state in surrounding tissues. If these responses are delayed, weak, or neutralized, viral replication increases and the risk of severe disease rises.
5. Mucosal IgA helps reduce viral establishment at the site of entry, neutralizing antibodies limit viral spread, and CD4/CD8 T cells support viral control and recovery. Together, they also contribute to immune memory.
6. Severe COVID-19 can show adaptive responses and lymphopenia at the same time because immune activity may still be present even when circulating lymphocyte numbers are reduced. Lymphopenia reflects broader immune disruption and severe systemic illness.
7. Early COVID-19 is relatively virus-driven because disease is shaped mainly by viral replication and early containment. Severe COVID-19 becomes increasingly host-response driven because inflammation, endothelial injury, and coagulation abnormalities become major drivers of pathology.
8. Failed early antiviral containment allows higher viral burden, spread to the alveoli, pulmonary injury, and inflammatory amplification. This can then lead to endothelial dysfunction, coagulation abnormalities, immunothrombosis, and severe systemic disease.

CHAPTER

4

DISEASE OUTCOMES & OPPORTUNISTIC INFECTIONS

DR. S





Chapter 4

DISEASE OUTCOMES & OPPORTUNISTIC INFECTIONS

Learning Goals

By the end of this section, learners should be able to:

1. Outline how COVID-19 progresses from mild upper respiratory infection to pneumonia, ARDS, and systemic complications.
2. Describe how early interferon responses help control infection and why failure of this response enables lower respiratory spread of infection
3. Explain how epithelial (type II pneumocyte) injury, surfactant loss and diffuse alveolar damage contribute to diminished gas exchange in severe COVID-19.
4. Discuss how endothelial dysfunction, immunological dysregulation, and microvascular thrombosis lead to hypoxemia and multi-organ involvement.
5. Interpret how immune dysregulation and opportunistic infections lead to severe disease outcomes

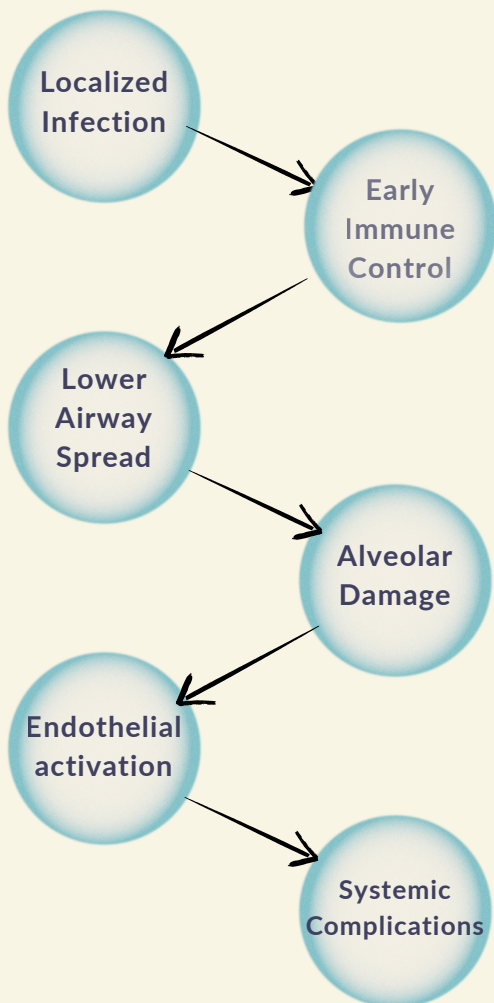
INTRODUCTION

COVID-19 presents with a wide spectrum of clinical manifestations, ranging from mild upper respiratory symptoms to severe pneumonia, ARDS, thrombosis, and multi-organ dysfunction. The pathogenesis of the disease is not dependent on the viral load alone; rather, the interaction between the viral replication, host immune response, and vascular injury^{20,21}. An important aspect of severe COVID-19 is that the dominant pathology evolves over time. In early stages, infection is driven by the viral infection, whereas in later stages, it is increasingly driven by host-mediated injury, which includes diffuse alveolar damage, immune system dysfunction, endothelial injury, and thrombosis^{21,23}. In this section, this progression is explained step by step in stages, starting from mild infection in the upper respiratory tract and concluding with systemic complications and opportunistic infections.

PROGRESSION OF DISEASE

COVID-19 can be viewed as a sequence of events:

- **Localized viral infection** in the upper respiratory tract
- **Early immune control** via interferon signaling
- Virus spread to the lower respiratory tract due to insufficient immune control
- **Alveolar injury** and compromise of gas exchange
- **Endothelial activation** and thrombosis
- **Systemic complications** and secondary infections²⁰⁻²²



Each stage builds on the one before it. Severe disease can therefore be understood as a progression from viral infection to host-driven tissue and vascular damage.

STAGE 1: MILD INFECTION IN THE UPPER AIRWAY

In mild COVID-19, infection is mainly localized to the **upper respiratory epithelium**. Intracellular **pattern recognition receptors**, including as **RIG-I**, **MDA5**, and **Toll-like receptors**, identify the presence of viral RNA in infected cells and initiate the **innate antiviral response**²¹.

Downstream signals from pattern recognition receptors promote production of **type I and type III interferons**, which are responsible for

Functions of interferons

- **limit viral replication**
- **protect nearby cells**
- **reduce spread to the lungs**

limiting the infection before it spreads to the lower respiratory tract. Interferons have no direct role in destruction of the virus. However, they activate expression of **interferon-stimulated genes** (ISGs), which inhibit **viral replication**, halt **viral protein synthesis**, and degrade **viral RNA**²¹.

When this response is rapid and efficient, the spread of the virus is restricted, hence the disease remains mild and more importantly, localized.

PRESERVED PULMONARY FUNCTION

The fact that mild disease does not cause any significant respiratory compromise can be explained by the presence of an intact **alveolar-capillary** membrane. At this stage, the structures involved in **gas exchange** have not yet sustained substantial damage²².

In mild disease:

- alveoli remain open and structurally preserved
- endothelial cells retain their normal **anticoagulant phenotype**
- inflammatory infiltration is restricted
- platelet activation is low
- **gas exchange** is still effective²²

This explains why **hypoxemia** typically does not develop in patients with mild disease. Despite the infection, the lung can maintain functional gas exchange, effectively transferring oxygen into the bloodstream.

STAGE 2: PROGRESSION TO PNEUMONIA

If early host antiviral control is delayed or inadequate, this allows SARS-CoV-2 virus to propagate into the lower respiratory tract and the bronchiole-alveolar region, where it infects alveolar epithelial cells, particularly **type II pneumocytes**^{21,24}.

Type II pneumocytes are crucial because they:

- synthesize **surfactant**, which decreases surface tension to allow alveoli to remain open and avoid their collapse
- act as progenitor cells that contribute to regeneration of damaged alveolar epithelium

Infection within such cells results in **apoptosis** and **necrosis**, leading to both decreased lung surfactant production and regeneration ability²⁴. This is a critical point because the infection is no longer contained, and becomes destructive to both lung structure and function.

Comparison of Normal and Infected Type II Pneumocytes

Feature	Normal type II pneumocyte	Infected type II pneumocyte
Surfactant	Produces and maintains surfactant	Low surfactant production
Alveolar Integrity	Supports alveolar integrity and stability	Increased cell death and loss of alveolar integrity
Repair	Contributes to repair	Impaired alveolar repair

SURFACTANT LOSS AND ATELECTASIS

As the infected type II pneumocytes suffer apoptosis and decline in number, **surfactant** production also falls. By lowering surface tension, surfactant agents normally prevent alveolar collapse. Hence, lack of surfactant causes the alveoli to become unstable and more susceptible to collapse, a process known as **atelectasis**²⁴.

Direct consequences for respiration:

- less air reaches the **gas-exchange surface**
- reduced functional alveolar surface area
- **oxygen diffusion** becomes less effective

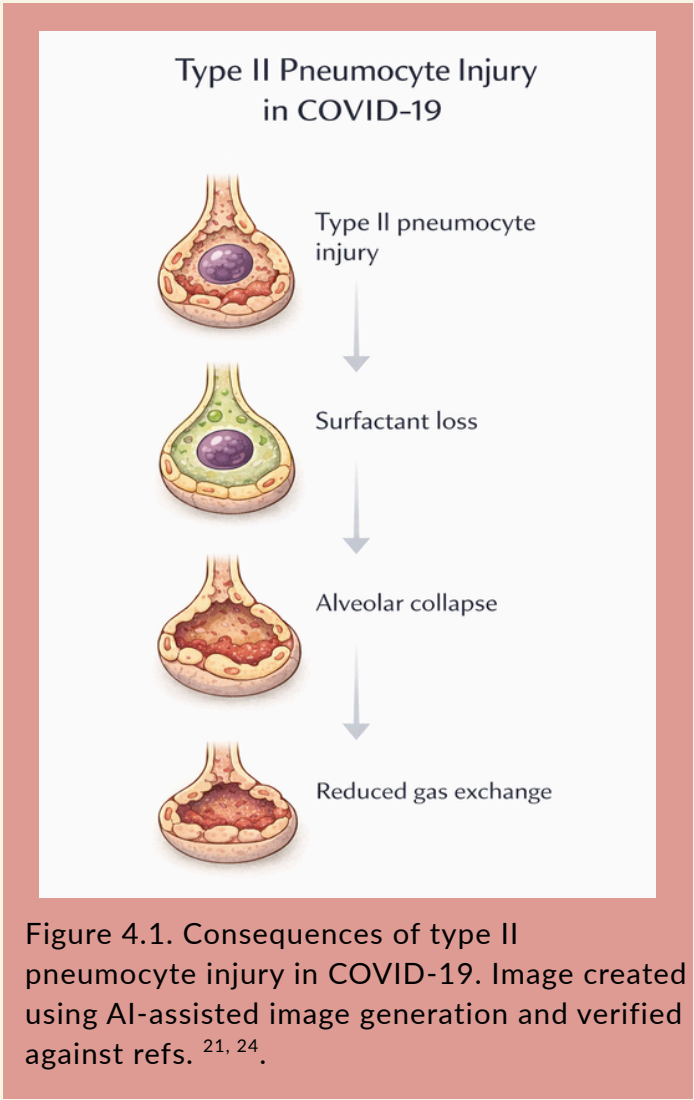


Figure 4.1. Consequences of type II pneumocyte injury in COVID-19. Image created using AI-assisted image generation and verified against refs. ^{21, 24}.

Overall, type II pneumocyte injury impairs **alveolar stability** and **gas exchange**, marking the transition from localized infection to structurally significant lung disease.

HOW LOCAL DAMAGE BECOMES DIFFUSE LUNG INJURY

As the infected pneumocytes **apoptosis**, they produce **damage-associated molecular patterns (DAMPs)** which recruit and activate inflammatory cells such as **alveolar macrophages, monocytes, and neutrophils**²¹.

Inflammation is also amplified when recruited inflammatory cells release pro-inflammatory chemical mediator cytokines such as **IL-6, TNF- α , and IL-1 β** . Although this inflammation response is meant to control the infection, it can equally cause damage to the host tissues as well.

The most relevant consequence is the disruption of **epithelial and endothelial barriers**, which results in increased permeability and fluid and plasma protein leakage into the alveolar spaces²⁵. This contributes to **pulmonary edema** and exacerbates **gas exchange**.

3 Underlying Mechanisms

Cell death and DAMP release

- Apoptotic pneumocytes release DAMPs that trigger local inflammation.

Cytokine release

- Inflammatory cytokines amplify local lung injury.

Barrier Disruption

- Fluid and plasma proteins enter alveoli, worsening gas exchange.

DIFFUSE ALVEOLAR DAMAGE (DAD)

Diffuse Alveolar Damage (DAD) is the hallmark histopathologic feature of severe COVID-19 infection²⁵. This reflects extensive damage to the **alveolar-capillary unit**.

Key features include:

- **alveolar edema**
- **epithelial injury**
- leakage of **proteinaceous fluid**
- **hyaline membrane formation**
- loss of normal alveolar architecture²⁵

Hyaline membranes fill with plasma proteins, fibrin and necrotic debris, which physically interfere with **oxygen diffusion** into tissues. Thus, severe COVID-19 is more than “inflamed lungs” – it is a condition characterized by disruption of the physical process of **gas exchange**.

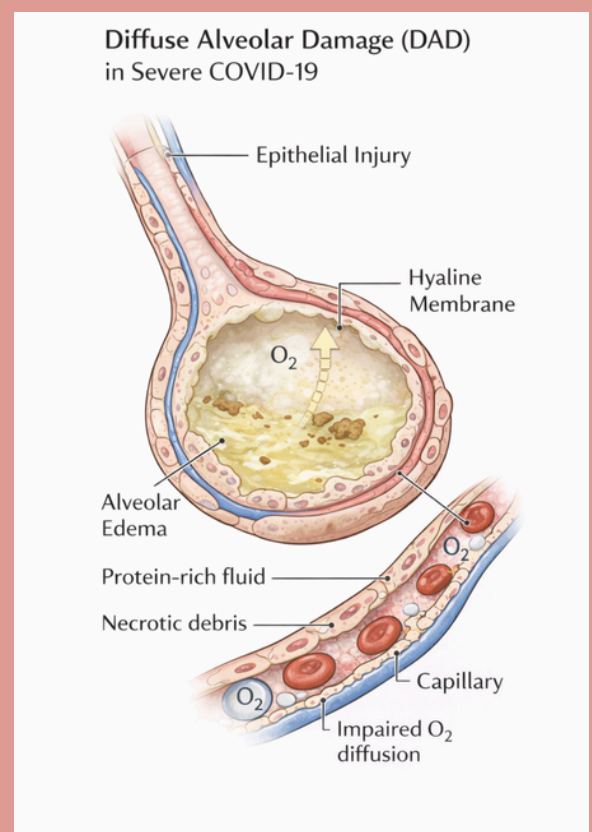


Figure 4.2. Structural features of diffuse alveolar damage in severe COVID-19, including edema, hyaline membrane formation, protein-rich alveolar fluid, and poor oxygen diffusion. Image created using AI-assisted image generation and verified against ref²⁵.

ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)

In COVID-19, **ARDS** is considered the most severe type of pulmonary damage. This pathology arises as a consequence of widespread **diffuse alveolar damage** lesions to the point where the alveolar-capillary interface fails^{22,25}.

Key features of ARDS include:

- **fluid-filled alveoli**
- reduced lung compliance
- severe impairment of **oxygen diffusion**
- **intrapulmonary shunting**^{22,25}

Bypassing lung areas with poor or nonexistent ventilation is known as **intrapulmonary shunting**. As a result, the blood's oxygen content is low when it exits the lungs. This highlights why ARDS patients experience severe **hypoxemia** and commonly rely on intensive respiratory support.

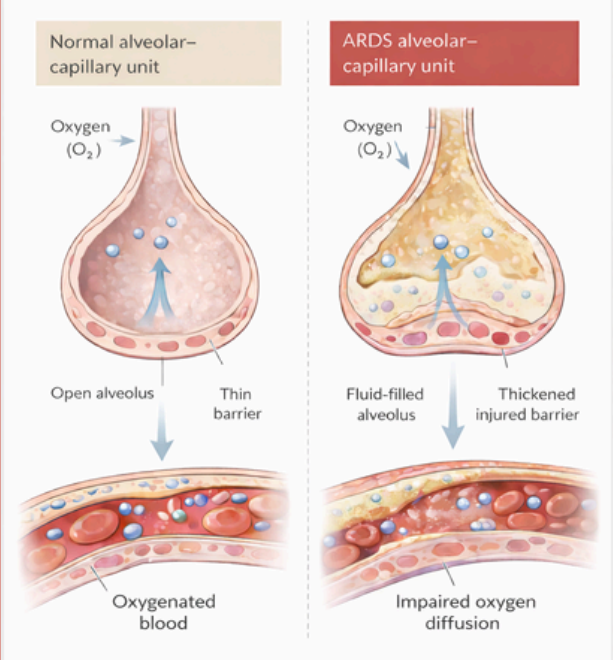


Figure 4.3. Comparison of a normal alveolar-capillary unit and an ARDS-affected unit in severe COVID-19, illustrating fluid-filled alveoli, barrier thickening, impaired oxygen diffusion, and reduced blood oxygenation. Image created using AI-assisted image generation and verified against refs.^{22, 25}.

VASCULAR INVOLVEMENT IN SEVERE COVID-19

A distinguishing feature of severe COVID-19 compared to other types of pneumonia is the degree of **vascular involvement**. In addition to epithelial damage, severe COVID-19 also causes damage to the **endothelium**, the cell layer lining blood vessels^{22,23}.

Normally, endothelial cells are involved in regulation of permeability, preventing inappropriate clotting, and maintaining **vascular homeostasis**. In severe COVID-19, inflammatory cytokines, immune-mediated injury, and complement activation promote **endothelial activation**^{22,23}.

The activated endothelial cell becomes increasingly inflammatory and procoagulant. Rather than creating an anticoagulant state within the vasculature, activated endothelium causes increased adhesion, inflammation, and **thrombus formation**^{22,23}.

Comparison of Normal and Activated Endothelium

Feature	Normal endothelium	Activated Endothelium
Vascular state	Maintains vascular stability	Promotes vascular dysfunction
Coagulation	Limits clot formation	Favors thrombosis
Permeability	Regulates permeability	Becomes more permeable
Inflammation	Limits leukocyte adhesion	Promotes Inflammation

FROM ENDOTHELIAL ACTIVATION TO CLOT FORMATION

Inflammation and coagulation start to reinforce one another when the **endothelium** is activated; this process is known as **thromboinflammation**^{22,23}.

Various factors are involved in this process, including the activation of the coagulation system, activation of **platelets**, decreased anticoagulation activity, and **neutrophil extracellular traps (NETs)**^{22,23}.

NETs are web-like DNA networks released by neutrophils to help trap pathogens. However, NETs can also serve as a structural basis for clot development and promote **microvascular thrombosis**.

In turn, this leads to formation of **microthrombi**, especially in the small vessels of the lungs (pulmonary capillaries), causing worse **perfusion** of the lungs and exacerbation of respiratory dysfunction²².

WHY MICROTHROMBI MATTER

The significance of **microthrombi** cannot be underestimated because oxygen transfer requires not only healthy alveoli that can perform the process but also sufficient blood flow to utilize for the process. Oxygenation is hampered if **capillary perfusion** is impaired.

This emphasizes that severe COVID-19 is more than just a **ventilation** problem. It is equally a vascular **perfusion** problem, where clots within the pulmonary microvasculature block effective oxygen uptake into circulation.

As thrombotic obstruction spreads and becomes more numerous, there is an increasingly large discrepancy between **ventilation** and **perfusion**, creating the conditions for intense **hypoxemia**.

WHY OXYGEN LEVELS DROP IN SEVERE COVID-19

Hypoxemia that occurs during severe COVID-19 is associated with dysfunction of the ventilation-perfusion relationship. Gas exchange surface area is reduced because some alveolar collapse, fill with fluid, or interact with hyaline membranes, while others get inadequate blood flow due to clots in the pulmonary microvasculature²²⁻²⁵.

4 Mechanisms of Hypoxemia

Ventilation defect

- Alveolar collapse, fluid accumulation, and hyaline membranes impair oxygen diffusion.

1

Perfusion defect

- Microthrombi reduce pulmonary capillary blood flow.

2

V/Q mismatch

- Airflow and blood flow become increasingly uncoupled.

3

Severe hypoxemia

- Combined ventilation and perfusion defects reduce effective oxygenation.

4

LABORATORY FINDINGS AND CLINICAL INTERPRETATION

By this stage of disease, the consequences of **endothelial dysfunction** and **microthrombi** are no longer only structural or mechanistic – they also become clinically measurable.

Laboratory abnormalities can be linked to microvascular thrombosis. One of the most significant is an elevated **D-dimer**, a product resulting from clot formation and subsequent dissolution²².

Other possible tests might look for higher **fibrin degradation products** due active clot turnover. Although these test results do not prove thrombosis on their own, they confirm the presence of **coagulation-fibrinolysis** processes during severe disease.

This helps pave the way for the next stage in this part, which will focus on how vessel and immunity problems can cause more than pulmonary complications.

Labratory Results Explained

Marker	Interpretation
D-dimer ↑	Active clot formation and breakdown
Fibrin degradation products ↑	Ongoing coagulation and fibrolysis

These findings help connect microvascular thrombosis to measurable laboratory evidence in severe COVID-19.

FROM IMMUNE DYSREGULATION TO SYSTEMIC DISEASE

Severe COVID-19 is associated with **immune dysregulation** and it is not simply a stronger version of mild disease. Delayed or insufficient early **interferon** response followed by continuous activation of the innate immunity is a typical pattern²¹.

As this response worsens, inflammatory mediators such as **IL-6**, **CRP**, and **ferritin** increase, reflecting an ongoing systemic inflammatory state²¹. On the other hand, there could be a failure of the adaptive immune system in the form of **lymphopenia** and decreased **CD4+** and **CD8+ T-cell** activity. As a result, the immune response becomes both hyper- inflammatory and incapable of restoring normal immune balance.

Importantly, severe COVID-19 does not always fit the classic definition of a cytokine storm. It should be noted that a cytokine storm does not always occur in severe forms of coronavirus infection. Even though the amount of cytokines is usually high, their level is typically significantly lower compared to the levels of cytokines in classical cases of a cytokine storm, which is why the term immune dysregulation is more accurate.

This altered immune state extends injury beyond the lungs. When inflammation and vascular dysfunction occur on a systemic level, organ damage may develop in multiple sites, including:

- **acute kidney injury**
- **myocardial injury**
- **cerebrovascular events**^{22,23}

Hence, severe cases of COVID-19 need to be considered not merely a respiratory ailment, but also an **inflammatory** and **vascular** disease involving several organ systems.

WHY SEVERE DISEASE INCREASES INFECTION RISK

Patients with severe COVID-19 are predisposed to acquiring **secondary infections**, both **bacterial** and **fungal**, due to a combination of factors.

First, **epithelial damage** compromises the barrier effects of the respiratory system. Second, severe illness often necessitates **hospitalization**, the use of invasive equipment, and **mechanical ventilation**, further increasing exposure to **nosocomial** pathogens. Third, some treatments such as **corticosteroids** and **immunomodulators** can cause suppression of **host immune function** and reduce host resistance to infection.

As a result, severe COVID-19 patients have high chances of developing secondary infections, including **bacterial pneumonia** and fungal infections such as **COVID-19-associated pulmonary aspergillosis** [8]. These infections are not coincidental, and directly take advantage of opportunities presented by **epithelial damage**, **immune dysregulation**, and the therapies used to manage severe disease.

Key factors increasing infection risk:

- **epithelial barrier disruption**
- **ICU exposure and mechanical ventilation**
- **immune dysregulation**
- **corticosteroid and immunomodulator use**

BRINGING IT ALL TOGETHER

Pathological progression of COVID-19 begins with **localized respiratory infection** but proceeds to become a disease of **damaged alveoli**, **immune dysregulation** and **vascular dysfunction**. These factors work in tandem to disrupt proper **gas exchange**, induce **microthrombosis**, create a favoring conditions for **secondary infections**, and ultimately drive **systemic complications**. The key takeaways list the major mechanisms linking viral infection to clinical outcomes.

Key Takeaways

- The progression of severe COVID-19 infection occurs in phases and involves the infection of localized regions moving towards lower respiratory tract involvement, diffuse alveolar damage, vascular injury, and eventually systemic complications.
- Type II pneumocyte damage marks a major turning point because of decreased surfactant synthesis, impairs alveolar repair, destabilizes alveoli, and exacerbates gas exchange.
- Diffuse alveolar damage (DAD) is one of the most common pathological features of severe COVID-19 lung infection and affects the alveolar-capillary interface through edema, epithelial damage, hyaline membrane formation, and impaired oxygen diffusion.
- ARDS leads to severe hypoxemia due to fluid-filled alveoli, decreased lung compliance, impaired diffusion, and intrapulmonary shunting together reduce effective oxygenation.
- Endothelial activation and microthrombosis worsen disease by progressing advancing inflammation into impaired pulmonary perfusion, increasing ventilation-perfusion mismatch and further reducing oxygen levels.
- COVID-19 is not solely an infection of the lungs but involves immune dysregulation, thromboinflammation, and barrier disruption that may drive multisystem involvement and predispose the patient to opportunistic infections.

TEST YOUR KNOWLEDGE

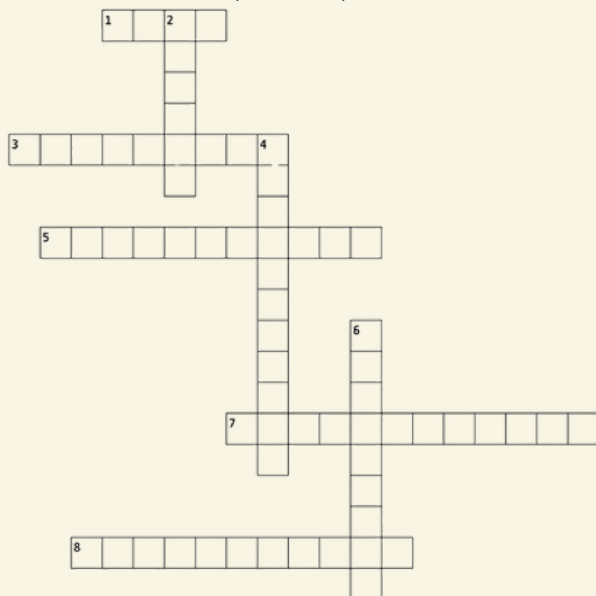
1. How does severe COVID-19 disease progress from a localized infection in the upper respiratory tract to diffuse alveolar damage, vascular dysfunction, and systemic disease?
2. Why is damage to type II pneumocytes considered a major turning point in disease progression? Include the significance of surfactant, alveolar stability, and alveolar repair.
3. What leads to severe hypoxemia in ARDS patients during severe COVID-19? Refer to fluid-filled alveoli, diffusion impairment, reduced compliance, and intrapulmonary shunting.
4. How does the activation of endothelium evolve into thromboinflammation in severe COVID-19? Discuss coagulation activation, NETs, and microthrombi.
5. Why can hypoxemia occur despite some of the alveoli being ventilated? Use the principle of ventilation-perfusion mismatch.
6. What does a high D-dimer indicate about the vascular pathology of severe COVID-19, and why is it of clinical importance?
7. Why do patients with severe COVID-19 have a greater susceptibility to secondary bacterial and fungal infections?

GAME

GAME:

SEVERE COVID-19 MECHANISMS REVIEW

Use the clues below to review key mechanisms and pathologic features of severe COVID-19



Across

1. Severe diffuse lung injury syndrome
3. Reduced oxygen in blood
5. Inner lining of blood vessels
7. Tiny clots in capillaries
8. Alveolar cells involved in surfactant production

Down

2. Marker of fibrin breakdown
4. Collapse of alveoli due to surfactant loss
6. Signalling molecules that amplify inflammation

Answer key below (upside down)

Down: 2. D-DIMER, 4. ATELECTASIS, 6. CYTOKINES
Across: 1. ARDS, 3. HYPOXEMIA, 5. ENDOTHELIUM,
7. MICROTHROMBI, 8. PNEUMOCYTES

ANSWERS

(Note answers need not be identical; provided below are model/sample answers)

1. Severe COVID-19 begins in the upper respiratory tract but can spread to the alveoli, where viral infection causes diffuse alveolar damage. This leads to inflammation, fluid accumulation, and endothelial dysfunction, which can then contribute to systemic disease.
2. Damage to type II pneumocytes is critical because they produce surfactant, which keeps alveoli open. Without surfactant, alveoli collapse, and since these cells also help with alveolar repair, their loss slows recovery.
3. Severe hypoxemia in ARDS occurs because alveoli become fluid-filled, impairing gas exchange. In addition, thickened membranes, reduced compliance, and intrapulmonary shunting all limit oxygen transfer to the blood.
4. Endothelial activation in severe COVID-19 promotes coagulation and inflammation. NETs (neutrophil extracellular traps) and clotting factors contribute to the formation of microthrombi, leading to thromboinflammation.
5. Hypoxemia can still occur due to ventilation-perfusion mismatch, where some alveoli are ventilated but not properly perfused, or blood is directed to damaged, non-ventilated areas.
6. A high D-dimer indicates increased fibrin breakdown, meaning there is active clot formation and degradation. This reflects hypercoagulability and is associated with more severe disease.
7. Patients with severe COVID-19 are more prone to secondary infections because of immune dysregulation, damage to the respiratory epithelium, and the use of immunosuppressive treatments like corticosteroids.

CHAPTER

5

DIAGNOSTICS, LAB
MEDICINE, & THERAPIES

DR. A





Chapter 5

DIAGNOSTICS, LAB MEDICINE, & THERAPIES

Learning Goals

By the end of this section, learners should be able to:

1. Identify the various laboratory techniques used to diagnose SARS-CoV-2 infection, and the limitations and strengths of each
2. Understand the application of Computed Tomography (CT) imaging in diagnosis and treatment of SARS-CoV-2 infection
3. Identify various therapies, such as antivirals, anticoagulants, and immunomodulators used in treatment
4. Describe how treatment is integrated with the active phase of disease
5. Understand the benefits and efficacy of vaccinations, and their overall role in preventing severe disease

INTRODUCTION

The effective and timely diagnosis of SARS-CoV-2 infections relies on a range of laboratory-based and adjunct techniques that detect either the presence of the virus, the host response to infection, or related radiological signature.

In clinical pathology, accurate interpretation of these tests requires an understanding not only of the underlying methodology but also of the biological context, including viral load, timing of infection, and specimen quality.

Diagnostic strategies therefore integrate multiple different detection modalities, with nucleic acid-based assays serving as the reference standard while other approaches provide complementary roles depending on clinical needs.

RT-PCR(REVERSE TRANSCRIPTION–POLYMERASE CHAIN REACTION)

RT-PCR is considered the gold standard for SARS-CoV-2 diagnosis due to its high analytical sensitivity and specificity. The method is based on the detection of viral RNA, which is first converted into complementary DNA (cDNA) via reverse transcription. This cDNA is then amplified through repeated cycles of PCR, causing exponential amplification of target sequences. Fluorescent probes are incorporated into the reaction, enabling real-time detection as signal intensity increases with each amplification cycle.²⁸

RT-PCR assays typically target conserved regions of the viral genome, including the nucleocapsid (N) gene, envelope (E) gene, and replication-associated regions such as RNA-dependent RNA polymerase (RdRp) or ORF1ab, which are genes crucial for viral replication.^{28,29}

An internal control, most commonly human RNase P, is included to confirm that the sample contains human material and that nucleic acid extraction has been successfully performed.

The diagnostic workflow begins with specimen collection, typically via nasopharyngeal swabs or saliva samples. Viral RNA is extracted from the sample, reverse transcribed into DNA, and amplified using PCR, with fluorescence monitored throughout the process.^{28,29}

A key parameter generated during this process is the cycle threshold (Ct) value, defined as the number of amplification cycles required for the fluorescent signal to exceed a predefined detection threshold. Lower Ct values indicate higher initial quantities of viral RNA, whereas higher Ct values reflect lower viral loads. However, it is important to note that Ct values are not standardized across laboratories, assay platforms, or instruments, limiting comparability.^{29,30}

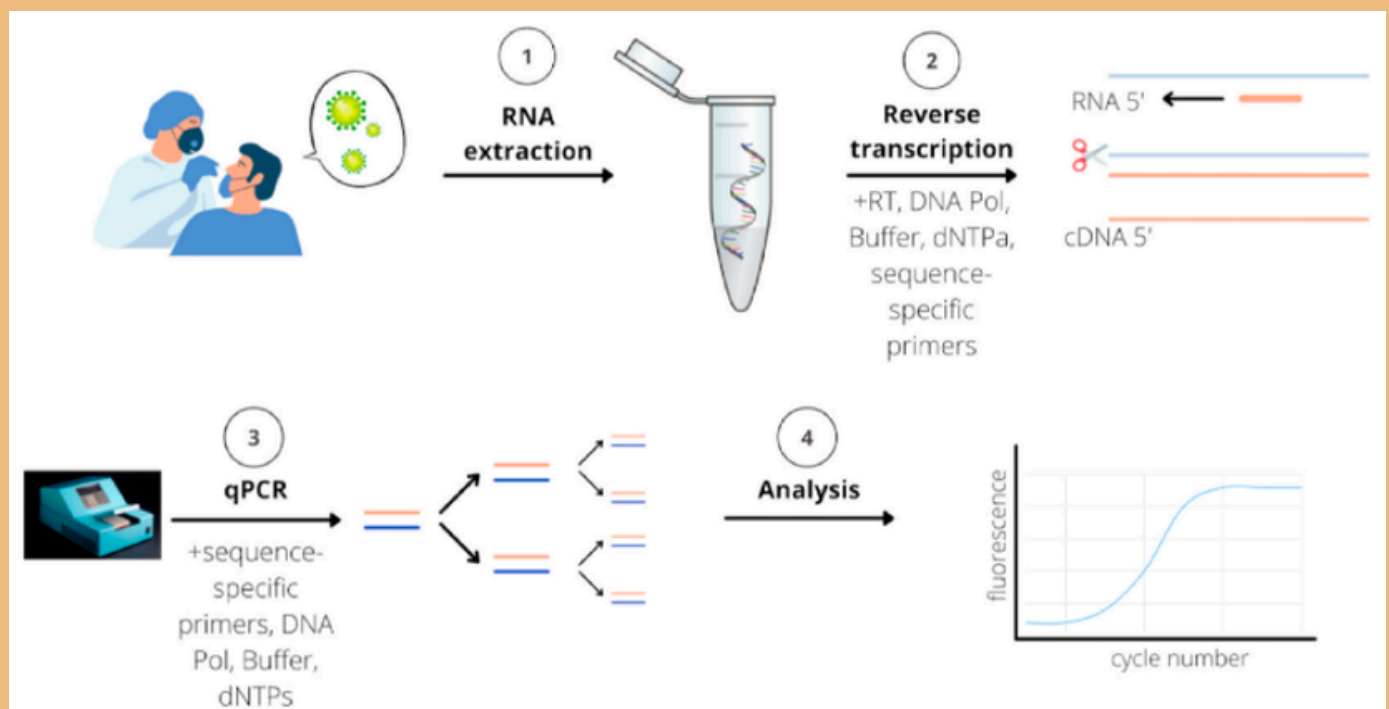


Figure 5.1. Diagnostic RT-PCR workflow showing extraction, reverse transcription, qPCR, and analysis. Image taken from Filchakova et al., 2022²⁸

RT-PCR offers very high sensitivity, allowing for detection of small samples of viral RNA, and achieves high specificity due to sequence-based targeting. Despite these strengths, the technique requires specialized laboratory infrastructure and trained personnel, and turnaround time may be influenced by logistical factors.^{26,27}

Additionally, false-negative results may occur due to poor sample collection, testing during early infection when viral load is below the detection threshold, or degradation of viral RNA. False-positive results can arise from contamination or nonspecific amplification at late cycles. Clinically, it is important to recognize that RT-PCR detects viral RNA rather than viable virus, meaning that RNA fragments may persist after the infectious phase has resolved. Consequently, Ct values should not be interpreted as direct measures of infectiousness or disease severity.^{26,27,28}

RT-LAMP (Reverse Transcription Loop-Mediated Isothermal Amplification)

RT-LAMP is an alternative nucleic acid amplification method that operates at a constant temperature, typically between 60 and 65°C. Unlike RT-PCR, which requires repeated temperature cycling, RT-LAMP uses a set of primers targeting multiple regions of the viral genome, enabling rapid isothermal amplification. While this multi-primer approach enhances specificity, it also increases the complexity of assay design.^{28,29,30}

In RT-LAMP, reverse transcription and amplification occur in a single reaction. Detection is typically achieved through either a colorimetric change or fluorescence. The absence of a requirement for a thermocycler also reduces equipment needs, making this method more accessible in settings with limited funding.^{28,30}

RT-LAMP provides results within approximately 20 to 30 minutes and is relatively low cost, supporting its use in high-throughput screening. However, its sensitivity is lower than that of RT-PCR, particularly at low viral loads associated with early stages of infection. Detection becomes less reliable in samples corresponding to higher Ct values in RT-PCR, generally above ~30, limiting its early effectiveness. Clinically, RT-LAMP is best used as a screening tool, with positive results often requiring confirmation by RT-PCR in settings where diagnostic certainty is a necessity.^{28,29}

CRISPR-Cas-Based Diagnostics

CRISPR-based diagnostic systems represent an emerging approach that utilizes CRISPR-associated enzymes such as Cas12 or Cas13 to detect viral nucleic acids with high specificity. These systems employ guide RNA to direct the enzyme to a specific viral sequence. Upon target recognition, the enzyme becomes activated and performs collateral cleavage activity, cutting nearby reporter molecules and generating a detectable signal.^{28,29}

Because viral RNA levels in clinical samples are often low, CRISPR-based detection is typically preceded by an amplification step. The workflow therefore involves RNA extraction, amplification of the target sequence, CRISPR-mediated detection, and signal readout via fluorescence or lateral-flow assays.²⁹

CRISPR-based assays offer extremely high sensitivity, with the potential to detect very few RNA copies. They are generally faster than conventional RT-PCR. As current implementations involve multiple steps and lack widespread standardization, routine use in clinical laboratories varies.²⁸

Antigen-Based Tests

Antigen-based tests detect viral proteins, most commonly the SARS-CoV-2 nucleocapsid protein, without the need for nucleic acid amplification. As a result, detection depends directly on the amount of virus present in the sample that is being tested.³⁰

These tests are characterized by their speed and simplicity, typically producing results within 10 to 15 minutes and requiring minimal equipment. They are also relatively inexpensive and therefore served as an accessible, rapid, and economical testing method during the peak of the COVID-19 pandemic.^{28,30}

The primary limitation of antigen testing is its lower sensitivity compared with nucleic acid-based methods. Detection is poor during early infection, when viral loads remain low, and during later stages when viral levels have declined.²⁸

Clinically, a positive antigen test is generally indicative of active infection. However, a negative result does not exclude infection and may require confirmation with a more sensitive method such as RT-PCR.³⁰

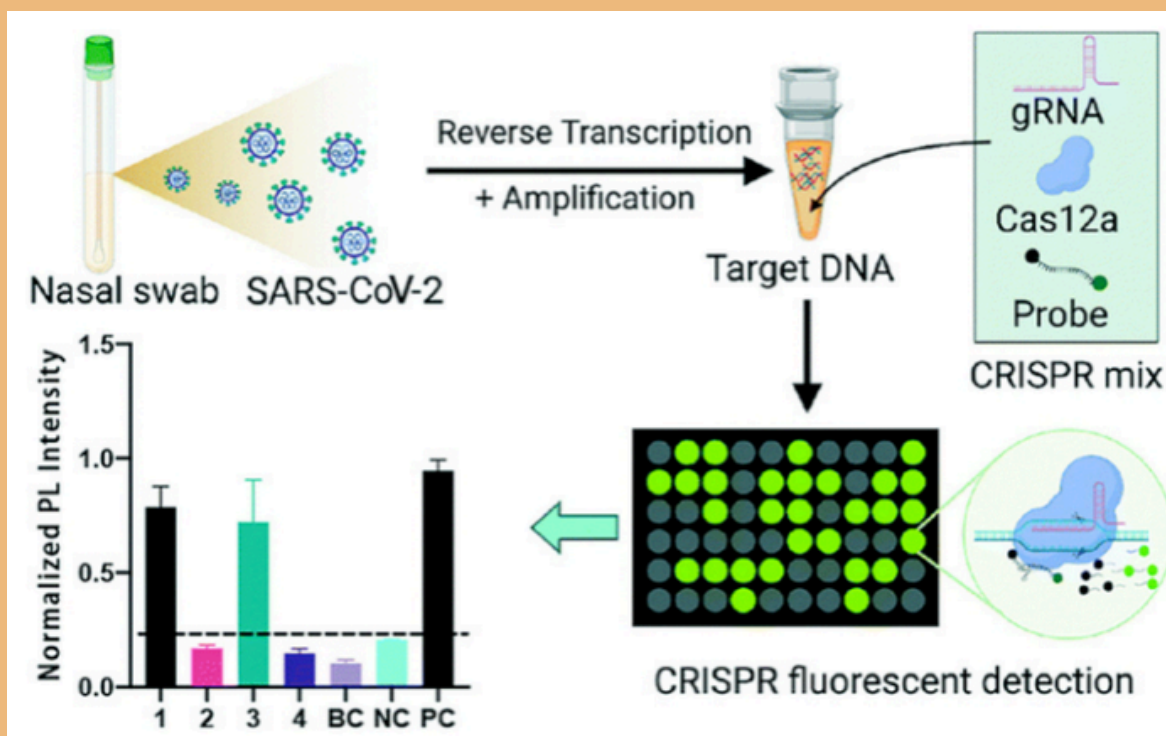


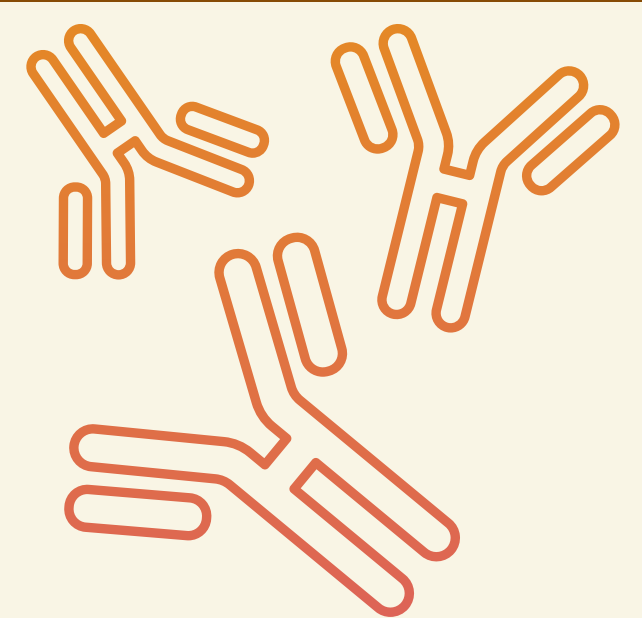
Figure 5.2. CRISPR-Cas based detection workflow. Image taken from Alyafei et al., 2022²⁹

Antibody (Serological) Tests

Serological tests detect the host immune response to SARS-CoV-2 rather than the virus itself. These assays typically measure immunoglobulin M (IgM), which may appear earlier but is variable, and immunoglobulin G (IgG), which develops later and persists for a longer duration.³⁰

Antibodies generally become detectable one to three or more weeks after infection, rendering serology unsuitable for diagnosing acute or early infection.²⁷ Instead, these tests are used to identify prior infection, assess seroprevalence, and evaluate immune responses. This data contributes heavily to the field of epidemiology and informs broader decisions and research, rather than serving as a direct technique to assess a current infection.^{29,30}

Limitations include the previously discussed inability to confirm active infection, the potential for false-negative results prior to seroconversion, and possible cross-reactivity with antibodies against other coronaviruses.^{29,30}



Method	Sensitivity	Speed	Infrastructure	Best Use
RT-PCR	Very high	Slow	High	Confirmation
RT-LAMP	Moderate-high	Fast	Low	Screening
CRISPR	Very high	Fast	Moderate	Rapid confirmation
Antigen	Low	Very fast	Minimal	High-load screening
Serology	N/A (active)	Moderate	Moderate	Past infection

Computed Tomography (CT) Imaging in COVID-19

Computed tomography (CT) provides a non-invasive imaging method for evaluating pulmonary involvement in COVID-19.

Unlike laboratory-based diagnostics, CT does not detect SARS-CoV-2 itself but instead identifies structural abnormalities within the lungs caused by the pathogenesis of the disease, as discussed in earlier sections.

Typical findings include ground-glass opacities, multifocal patchy consolidation, and the so-called “crazy-paving” pattern, which is marked by ground-glass opacities in conjunction with septal thickening.^{30,31}

These findings evolve over time, with ground-glass opacities appearing within the first four days of infection, followed by increased involvement and development of the “crazy-paving” pattern between days five and eight.^{28,30}

Consolidation tends to predominate between days nine and thirteen, with gradual resolution occurring after approximately two weeks. Peak pulmonary involvement generally occurs around ten days after initial symptom onset.³⁰

CT imaging has several relevant limitations. First, sensitivity is reduced in early disease, with a substantial proportion of patients presenting with normal CT scans shortly after symptom onset.³⁰ Specificity is also limited, as CT findings overlap with influenza and other viral pneumonias and inflammatory lung diseases.²⁸

Discrepancies between RT-PCR and CT results may also occur; RT-PCR-negative but CT-positive findings may reflect sampling error or low viral load, whereas RT-PCR-positive but CT-negative cases often represent early infection or an absence of pulmonary involvement.³⁰

Clinically, CT is not used to definitively diagnose an active COVID-19 infection, but rather serves as an adjunct technique for assessing disease extent, monitoring progression, and supporting diagnosis when clinical suspicion remains high despite negative molecular testing.



Figure 5.3. CT scans showing the presence of ground-glass opacities (top), and their subsequent resolution following antiviral treatment (bottom). Image cropped from Marangoni et al., 2023.³¹

THERAPEUTIC APPROACHES FOR SARS-COV-2

Direct-Acting Antivirals

Direct-acting antivirals target viral replication and are most effective when administered early in infection, prior to the development of significant inflammatory responses. Their efficacy is closely linked to viral replication dynamics.^{31,32}

Remdesivir acts as an inhibitor of RNA-dependent RNA polymerase, reducing viral replication. It is most effective in hospitalized patients requiring oxygen, although its limited mortality benefit reflects the fact that many patients present at late, immune-driven stages of disease.^{32,33}

Molnupiravir induces lethal mutagenesis in viral RNA, leading to accumulation of errors that impair viral replication. Its effectiveness depends on early administration, and there are theoretical concerns regarding mutagenic effects on both viral and host genetic material.^{31,32,33}

Paxlovid, a combination of nirmatrelvir and ritonavir, inhibits the viral 3CL protease, preventing proper processing of essential viral proteins and therefore limiting replication. It is highly effective when used as an early treatment but is associated with significant drug-drug interactions due to the presence of ritonavir.^{31,32,35}

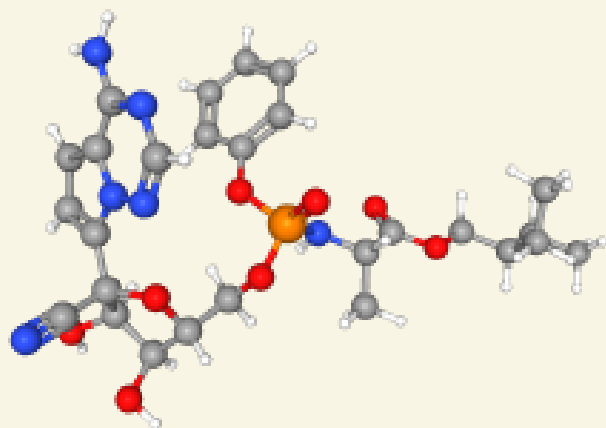


Figure 5.4. Pubchem structure of Remdesivir.³³



Figure 5.5. Pubchem structure of Molnupiravir.³⁴

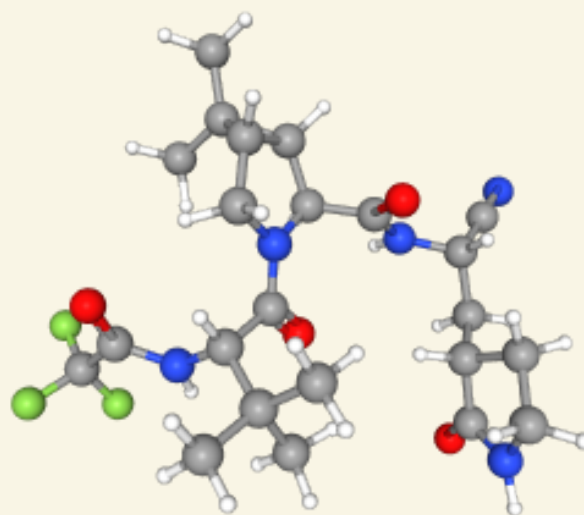


Figure 5.6. Pubchem structure of Nirmatrelvir.³⁵

These antivirals are most beneficial when viral load is high and inflammation is limited.³¹

As disease progresses and becomes immune-mediated, their effectiveness declines, and resistance may emerge through mutations in viral targets. This can necessitate the use of immunomodulators, as will be examined in the next section of this chapter.³²

Immunomodulators and Immunotherapies

Immunomodulatory therapies are designed to target dysregulated host immune responses and are most relevant in moderate to severe disease.

Corticosteroids such as dexamethasone exert broad immunosuppressive effects, reducing cytokine-mediated lung injury and improving outcomes in patients requiring oxygen or mechanical ventilation. However, they also increase the risk of secondary infections by limiting host immune response.³²

Interleukin-6 inhibitors, such as tocilizumab, block IL-6 signaling and are used to target cytokine storm physiology. Their benefit depends on the presence of elevated inflammatory markers, such as C-reactive protein.³²

Monoclonal antibodies neutralize the SARS-CoV-2 spike protein and prevent viral entry into host cells. Their effectiveness is highly dependent on viral variants, as mutations in the spike protein structure can significantly reduce binding and therefore efficacy. This highlights the need for broadly-acting antibodies.³²

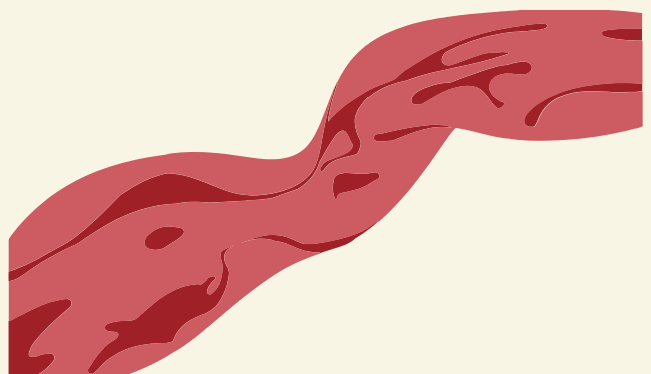
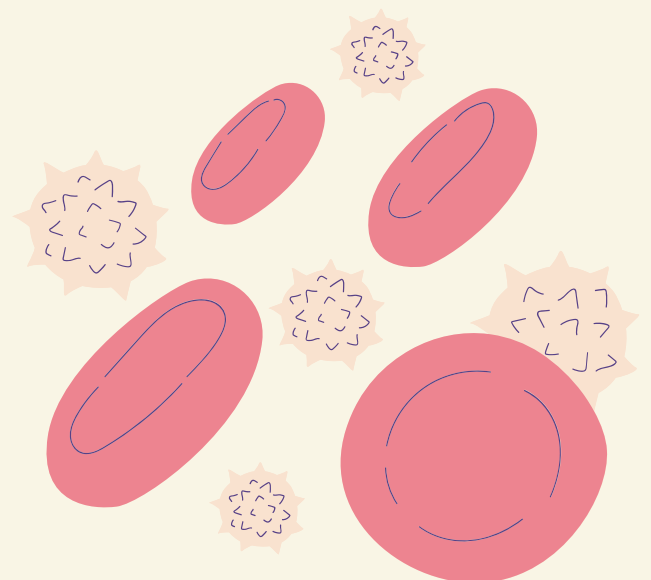
A key principle is that immunomodulators may be harmful if administered too early, when viral replication remains the dominant process. Their use should therefore be carefully guided by markers of host inflammatory response rather than by viral detection alone.³²

Anticoagulant Therapy

COVID-19 is also associated with a hyper-coagulable state driven by endothelial injury, inflammation, and impaired fibrinolysis, resulting in an increased risk of thrombotic events.³²

Laboratory findings commonly include elevated D-dimer and fibrinogen levels, reflecting increased coagulation activity. In many hospitalized patients, who are further susceptible due to intubation and the sedentary nature of inpatient care, the risk of thrombosis may exceed the risk of bleeding.³²

Prophylactic anticoagulation is standard in hospitalized patients, while therapeutic anticoagulation may provide benefit in selected non-intensive care unit patients. Laboratory markers are essential for risk stratification and management, requiring careful balancing of thrombotic and bleeding risks according to varying patient conditions.



Disease Phase	Dominant Process	Treatment Approach
Early Infection	Viral replication	Antiviral therapies
Intermediate Stage	Viral + immune response	Combination therapy
Late/Severe Disease	Immune-mediated injury	Corticosteroids, IL-6 inhibitors, and other immunomodulators
Hospitalized Patients (All Stages)	Coagulopathy risk	Anticoagulants + management of clotting disorders

VACCINATIONS FOR SARS-COV-2

COVID-19 vaccines are designed to prevent infection and, more importantly, to reduce the risk of severe disease, hospitalization, and death. They are not used as treatments for active infection.³⁶

Several vaccine platforms have been developed, including mRNA vaccines that deliver genetic material encoding the spike protein, viral vector vaccines that use non-replicating adenoviruses to deliver the spike gene, inactivated virus vaccines containing chemically inactivated whole virus, and protein subunit vaccines composed of purified viral proteins with adjuvants.³⁶

Vaccination induces both humoral and cellular immune responses. Neutralizing antibodies are generated against the spike protein, preventing viral entry, while CD4⁺ and CD8⁺ T cells contribute to cellular immunity. Over time, antibody levels naturally decline, reducing immunity.³⁶

Vaccines provide the strongest protection against severe disease and death. Vaccines are also shown to be beneficial in reducing the incidence of long COVID.³⁷ However, their effectiveness against infection is reduced with emerging variants. Booster doses restore antibody levels and enhance protective immunity.³⁶

COVID-19 vaccines are generally safe across platforms. Common adverse effects include injection-site pain, fatigue, and fever, while serious adverse events are rare.³⁶

Key Takeaways

COVID-19 diagnosis and management require an integrated approach.

Laboratory testing, imaging, and treatment strategies must be considered together.

RT-PCR remains the reference standard.

It is the most reliable method for detecting SARS-CoV-2 infection.

RT-PCR is supported by complimentary testing methods.

Antigen testing and serology play an important adjunct role.

CT imaging is used selectively.

It helps assess disease severity rather than simply confirm infection.

Treatment must match disease stage.

Management should reflect the shift from viral replication to host-driven pathology.

Vaccination is the most effective preventive measure.

It significantly reduces the risk of severe disease and complications.

All components work together in clinical care.

They form a comprehensive framework for managing COVID-19 infections.

TEST YOUR KNOWLEDGE

1. Why is RT-PCR considered the gold standard for diagnosing SARS-CoV-2 infections, and what is the basic workflow?
2. How does the timing of infection affect the sensitivity of antigen tests compared with RT-PCR?
3. Why are serology (antibody) tests not useful for diagnosing acute COVID-19 infections? What is the main use of serology testing?
4. How does CT imaging help assess the severity of COVID-19, even though it cannot confirm infection?
5. During which stage of COVID-19 are antivirals most effective, and why?
6. When are immunomodulators like corticosteroids or IL-6 inhibitors most beneficial in COVID-19 treatment?
7. Why is anticoagulation often necessary in hospitalized COVID-19 patients?

GAME



ANSWERS

(Note answers need not be identical; provided below are model/sample answers)

1. RT-PCR is the gold standard because it has very high sensitivity and specificity by detecting viral RNA directly through amplification. The basic workflow is sample collection followed by RNA extraction reverse transcription into cDNA and PCR amplification with real-time fluorescent detection.
2. Antigen test sensitivity depends strongly on timing because they require high viral load so they perform poorly early and late in infection while RT-PCR remains sensitive even at low viral levels.
3. Serology is not useful for acute diagnosis because antibodies take one to three weeks to develop. Its main use is identifying past infection and assessing immune response in populations.
4. CT imaging helps assess severity by showing lung damage such as ground-glass opacities and consolidation which reflect disease progression even though these findings are not specific to COVID-19.
5. Antivirals are most effective in the early stage of infection because viral replication is highest before significant immune-mediated damage occurs.
6. Immunomodulators are most beneficial in later moderate to severe stages when disease is driven by an excessive inflammatory response rather than viral replication.
7. Anticoagulation is often necessary because COVID-19 creates a hypercoagulable state that increases the risk of thrombosis especially in hospitalized and immobilized patients.

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