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Narrative Review

COVID-19: An updated comprehensive review

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Abstract

More than six years after the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), coronavirus disease 2019 (COVID-19) remains a complex global health challenge shaped by continued viral evolution, heterogeneous population immunity, and unequal access to prevention and care. The transition from the acute pandemic phase has changed the relationship between infection, hospitalization, and mortality, while reduced testing and surveillance complicate assessment of the contemporary burden. Ongoing diversification of Omicron descendants, waning protection against infection, and recurrent exposure have introduced additional uncertainties regarding transmissibility, immune escape, and clinical risk. Beyond acute respiratory illness, COVID-19 encompasses multisystem complications and persistent postinfectious symptoms, requiring an integrated appraisal of virological, immunological, clinical, and public health evidence. The aim of this narrative review is to critically synthesize current knowledge of SARS-CoV-2 epidemiology, genomic evolution, transmission, pathogenesis, host immune responses, and the spectrum of clinical manifestations. It also examines advances and limitations in diagnostic testing, severity-stratified therapeutics, vaccination, nonpharmaceutical interventions, and the recognition and management of long COVID. Particular attention is given to evidence gaps surrounding reinfection, immune durability, variant-specific risk, postacute disease mechanisms, equitable access to countermeasures, and sustainable surveillance. By connecting these domains, the review seeks to provide an updated framework for interpreting COVID-19 evidence and identifying priorities for clinical practice, public health policy, and future research.

Keywords: SARS-CoV-2, viral evolution, immune escape, post-COVID-19 condition, pandemic preparedness

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Introduction

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in late 2019 initiated an unprecedented global infectious disease crisis and fundamentally altered contemporary approaches to pandemic preparedness, clinical care, and biomedical research. Initially recognized through clusters of pneumonia of unknown etiology in Wuhan, China, the causative agent was subsequently identified as a previously unrecognized betacoronavirus. Early investigations established its genomic characteristics, association with respiratory disease, and

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capacity for sustained human transmission [1,2]. Coronavirus disease 2019 (COVID-19) rapidly progressed from a localized outbreak to a geographically extensive epidemic, facilitated by international mobility, efficient respiratory transmission, and infectiousness occurring before or in the absence of clinically apparent symptoms [3].

The early pandemic was characterized by profound uncertainty regarding the natural history of infection, determinants of clinical deterioration, optimal diagnostic approaches, and effective treatment. Although the respiratory tract was initially regarded as the principal site of disease, observations from hospitalized cohorts revealed a heterogeneous syndrome involving pulmonary, cardiovascular, renal, neurological, hematological, and immunological abnormalities [2]. The magnitude of the resulting health burden was amplified by overwhelmed healthcare systems, interruptions to essential services, delayed access to care, and marked inequities in diagnostic capacity and treatment availability. Consequently, the direct effects of viral infection cannot be fully disentangled from the broader indirect consequences of the pandemic when evaluating its population-level impact [4].

Alongside these clinical and health-system challenges, the epidemiology of COVID-19 has undergone substantial transformation since its emergence. During the initial pandemic years, high susceptibility in immunologically naive populations, limited availability of targeted preventive interventions, and repeated epidemic waves contributed to substantial excess mortality. A systematic analysis estimated 18.2 million excess deaths globally during 2020–2021 (95% uncertainty interval 17.1–19.6 million), greatly exceeding contemporaneously reported COVID-19 deaths [4]. Although excess mortality is not synonymous with deaths directly attributable to SARS-CoV-2 infection, this disparity underscores the limitations of routine notification systems and the multidimensional effects of the pandemic. The distribution of this burden was highly heterogeneous, reflecting differences in age structure, socioeconomic conditions, healthcare resources, surveillance completeness, and the timing and intensity of epidemic waves.

The termination of COVID-19 as a Public Health Emergency of International Concern in May 2023 represented an important transition in international public health governance, but did not signify elimination of SARS-CoV-2 or the cessation of COVID-19-associated morbidity and mortality [5]. Subsequent transmission has occurred within populations characterized by complex and changing immune histories arising from vaccination, previous infection, or both. Accordingly, the relationship between infection incidence and severe outcomes is no longer equivalent to that observed in 2020. Nevertheless, susceptible individuals remain at risk of hospitalization and death, particularly older adults, persons with substantial comorbidity, and those with impaired immune responses. The epidemiological interpretation of recent trends is further complicated by reduced diagnostic testing, variable case ascertainment, and heterogeneity in national surveillance systems.

An additional challenge is that recurrent infection may contribute to cumulative morbidity even where most acute episodes do not require hospitalization. The clinical and population-level consequences of repeated exposure require interpretation in the context of variant-specific properties, prior immunity, access to early treatment, and the underlying health status of affected individuals. Therefore, contemporary COVID-19 control is increasingly concerned not only with the prevention of acute mortality, but also with limiting severe outcomes, protecting vulnerable populations, preserving healthcare capacity, and understanding longer-term morbidity.

Addressing these evolving challenges requires an understanding of the virological and evolutionary processes that shape SARS-CoV-2 infection and reinfection. Unprecedented progress in virology and molecular epidemiology has clarified the biology and evolutionary potential of SARS-CoV-2. Structural investigations identified the central role of the spike glycoprotein in receptor engagement and viral entry, while large-scale genomic sequencing enabled the rapid detection and characterization of variants with distinct phenotypic properties [6]. Successive waves of viral evolution have altered antigenicity, receptor-binding characteristics, transmissibility, and susceptibility to antibody-mediated neutralization. In particular, the diversification of Omicron and its descendants has demonstrated the capacity of SARS-CoV-2 to persist through ongoing adaptation in populations with substantial pre-existing immunity [7]. These developments have important implications for interpreting vaccine

effectiveness, the performance of antibody-based interventions, and the durability of population-level protection.

Advances in transmission science have also reshaped preventive strategies. A growing body of mechanistic and epidemiological evidence has established the importance of inhalation of infectious respiratory particles, particularly in shared indoor air, thereby strengthening the scientific rationale for ventilation, respiratory protection, and layered infection prevention measures [3]. Concurrently, rapid progress in molecular diagnostics and genomic surveillance permitted earlier case identification and near-real-time monitoring of viral genetic diversity. However, unequal access to these technologies has continued to constrain the representativeness of the global evidence base.

The development and evaluation of vaccines represent one of the most consequential achievements of the pandemic response. Large randomized trials established the efficacy of messenger RNA vaccines against symptomatic COVID-19 during the early vaccine era [8]. Subsequent observational evidence demonstrated that protection against infection and protection against severe outcomes should be considered distinct endpoints, because both are affected by the circulating variant, time since immunization, prior infection, and host characteristics [9]. As viral antigenicity evolved, vaccine strategies increasingly required adaptation to changing epidemiological conditions rather than reliance on assumptions derived from the ancestral-virus period. The interaction between infection-induced and vaccine-induced immunity, commonly termed hybrid immunity, has consequently become central to understanding contemporary patterns of susceptibility and disease severity.

Therapeutic management has likewise evolved from predominantly supportive and empirically selected interventions toward evidence-based, severity-stratified care. Antiviral agents, particularly when administered early to appropriately selected patients, have expanded outpatient treatment options, while systemic corticosteroids and selected immunomodulatory therapies have improved management of severe inflammatory disease. Nevertheless, therapeutic benefits are context-dependent and cannot be generalized across disease severities or patient populations. Challenges remain regarding equitable access, timely initiation, contraindications, drug interactions, resistance, and the changing absolute benefit of treatment as baseline population risk evolves. These advances illustrate the necessity of periodically reassessing treatment recommendations against contemporary clinical trial and real-world evidence [10,11].

Despite advances in preventing severe disease and improving acute clinical outcomes, the consequences of SARS-CoV-2 infection may extend well beyond the initial illness. Recognition of post-COVID-19 condition, commonly referred to as long COVID, has expanded the conceptual framework of COVID-19 beyond the acute infectious episode. A consensus clinical definition developed through a World Health Organization-led Delphi process described a condition usually occurring three months after infection, involving symptoms persisting for at least two months that cannot be explained by an alternative diagnosis [12]. Common manifestations include fatigue, exertional intolerance, cognitive difficulties, dysautonomia, and respiratory symptoms, frequently accompanied by impaired physical function and quality of life. The heterogeneity of clinical presentations suggests that a single mechanism is unlikely to explain all affected individuals.

Proposed pathogenic pathways include dysregulated immune responses, persistent viral components or reservoirs, endothelial and microvascular abnormalities, autonomic dysfunction, and alterations in tissue repair processes. However, the relative contribution of these mechanisms remains incompletely characterized, and no single biomarker or therapeutic intervention has been established for the full spectrum of the condition [13]. Furthermore, prevalence estimates are sensitive to case definitions, follow-up duration, the inclusion of appropriate comparison populations, vaccination history, and differences among circulating variants. Long COVID therefore represents both a substantive clinical challenge and an important methodological problem for epidemiological research, with implications extending to workforce participation, disability, healthcare utilization, and health equity.

Other unresolved questions concern the future evolutionary trajectory of SARS-CoV-2, the clinical relevance of immune imprinting, the breadth and durability of mucosal and systemic immunity, and the potential consequences of successive reinfections. Sustained viral circulation creates continuing opportunities for evolutionary diversification, whereas declining routine

surveillance may delay the recognition of meaningful changes in viral phenotype. At the same time, differential access to updated vaccination, antiviral treatment, and specialist post-acute care may reinforce existing inequalities between and within countries [7,9,12]. These interconnected challenges necessitate an integrated understanding of viral biology, epidemiology, clinical manifestations, and preventive strategies.

Despite the extensive scientific literature addressing these challenges, the evidence base remains dynamic and increasingly fragmented across virology, immunology, epidemiology, clinical medicine, and public health. Reviews centered on the earliest pandemic waves may not adequately represent the implications of population immunity, continued evolution of Omicron descendants, changes in clinical risk profiles, advances in targeted treatments, or the burden of post-acute sequelae. Conversely, narrowly focused syntheses may provide limited insight into the relationships linking molecular evolution, immune protection, clinical outcomes, and public health interventions. A contemporary comprehensive review is therefore warranted to integrate these developments into a coherent scientific and clinical framework.

This review aims to critically synthesize the current understanding of COVID-19, from its changing epidemiology and the virological characteristics of SARS-CoV-2 to transmission dynamics, host-pathogen interactions, clinical manifestations, diagnosis, treatment, long-term consequences, and prevention. Particular attention is given to advances that have modified established knowledge, areas in which findings remain inconsistent, and the applicability of available evidence across diverse healthcare settings. By integrating mechanistic, clinical, and population-level perspectives, the review seeks to delineate the remaining scientific uncertainties and identify priorities for clinical practice, public health policy, and future research.

Epidemiology of COVID-19

Global epidemiological trends and disease burden

The global epidemiology of coronavirus disease 2019 (COVID-19) has been characterized by successive transitions in population susceptibility, viral fitness, clinical severity, and the ascertainment of infections. During the initial phase, widespread susceptibility to SARS-CoV-2 allowed explosive transmission, whereas case detection was constrained by laboratory capacity, restricted testing criteria, and substantial asymptomatic and mildly symptomatic infection. Consequently, the cumulative number of laboratory-confirmed cases represents only a subset of infections and cannot be interpreted as the true cumulative incidence. A systematic synthesis of population-based serosurveys demonstrated marked expansion of SARS-CoV-2 seropositivity during 2020–2022 and substantial underascertainment by routine testing systems [14]. Serological estimates, however, require distinction between antibodies attributable to infection and those induced by vaccination, particularly after widespread vaccine introduction.

COVID-19 also produced an exceptional global mortality burden, although its magnitude depends on the epidemiological indicator selected. The Global Burden of Disease (GBD) analysis estimated approximately 18.2 million excess deaths (95% uncertainty interval (UI): 17.1–19.6 million) during 2020–2021, compared with 5.94 million deaths officially reported as COVID-19 during the same period [4]. Separately, a World Health Organization (WHO) modeling analysis estimated 14.83 million excess deaths globally in 2020–2021, approximately 2.74 times the corresponding 5.42 million reported COVID-19 deaths [15]. These estimates should not be treated as interchangeable: their differences reflect underlying mortality inputs, model specifications, time-series assumptions, and uncertainty propagation. Furthermore, excess mortality encompasses direct effects of SARS-CoV-2 infection as well as indirect consequences of disrupted healthcare delivery and other pandemic-associated changes in mortality; it cannot be equated with virologically confirmed COVID-19 deaths.

Cause-specific estimates provide a complementary perspective. The GBD 2021 cause-of-death analysis estimated 4.80 million (95% UI 4.56–5.11 million) deaths attributable to COVID-19 in 2020 and 7.89 million (95% UI 7.49–8.40 million) in 2021 [16]. COVID-19 also interrupted decades of improvement in population survival: demographic estimates from 204 countries and territories showed substantial deterioration in global life expectancy during the acute pandemic period [17]. These findings demonstrate that the burden extended beyond the death counts

observed in routine clinical surveillance. Nevertheless, retrospective modeling remains sensitive to the completeness of civil registration and vital statistics, and regional uncertainty is generally greatest where mortality systems have been weakest.

By the period following the international public health emergency, the relationship between infections, hospital admissions, and mortality had become progressively less stable. Infection-acquired, vaccine-induced, and hybrid immunity; changes in circulating variants; earlier access to treatment; and changes in exposure patterns altered the probability that a given infection would produce severe disease. Simultaneously, discontinuation of mass testing and reductions in mandatory reporting substantially weakened the interpretation of trends in notified cases. Contemporary burden assessment should therefore triangulate severe acute respiratory infection surveillance, hospitalizations attributable to COVID-19, all-cause mortality, representative serological or community surveys, and environmental SARS-CoV-2 measurements rather than relying on any single epidemiological series.

Geographical distribution and regional disparities

Considerable spatial heterogeneity has persisted throughout the pandemic, with important differences between and within countries in exposure probability, case ascertainment, age structure, immunity, and healthcare access. During the early pandemic, seroprevalence frequently diverged from officially reported cumulative incidence. A systematic review of 968 seroprevalence studies involving approximately 9.3 million participants across 74 countries identified substantial geographical differences and found that nationally representative seroprevalence estimates were often many times higher than corresponding reported case incidence [18]. Such variation reflected not only genuine differences in transmission intensity but also unequal access to diagnostic testing and variation in study design, sampling frames, assay performance, and timing.

Differences in mortality burden similarly cannot be interpreted from absolute national death counts alone. Comparisons require at least age-standardized rates, the observation period, population denominators, ascertainment criteria, and uncertainty intervals. The global excess-mortality analysis identified particularly large estimated burdens in South Asia, North Africa and the Middle East, and eastern Europe, although the reliability of location-specific estimates varied with the availability of underlying mortality data [4]. Differences between estimated and recorded deaths are especially consequential when comparisons are made across health systems with substantially different diagnostic and death-certification capacities. Accordingly, a low reported case-fatality ratio may indicate extensive detection of mild disease, but it may also arise from systematic non-registration of deaths or changes in who is being tested.

Vaccine distribution introduced an additional dimension of geographical inequality. A global modeling analysis estimated that vaccination averted 19.8 million (95% credible interval 19.1–20.4 million) deaths in the first year of vaccine implementation when excess mortality was used as the outcome measure [19]. The same analysis estimated that unmet vaccination coverage targets in low-income countries were associated with a substantial opportunity to avert additional deaths [19]. These figures are modeled counterfactual estimates, not observed counts of prevented deaths; they nevertheless illustrate how differential access to immunization contributed to differences in the subsequent burden of severe disease. Beyond vaccine supply, deployment capacity, risk communication, vaccine acceptance, cold-chain infrastructure, and continuity of routine health services influenced the population-level benefits achieved.

Within countries, urban–rural differences, intergenerational household structures, labor-market conditions, mobility, and the distribution of long-term care facilities further modified transmission and outcomes. Spatial clustering in reported disease should not automatically be interpreted as clustering in infection risk: underserved communities may be less likely to obtain confirmatory testing or hospital care. High-resolution epidemiological comparisons are therefore strengthened by adjustment for age, deprivation, baseline comorbidity, testing availability, vaccination, and access to care, while ecological associations should not be extrapolated uncritically to individual-level causal effects.

Temporal changes in incidence, hospitalization, and mortality

The temporal course of COVID-19 can be understood as a sequence of overlapping epidemiological regimes shaped by viral replacement and changing population immunity rather than as a uniform trajectory of declining pathogenicity. Early epidemic waves were driven by the ancestral virus and subsequently by variants with altered transmission advantages, including Alpha and Delta. Omicron, first identified in late 2021, was associated with rapid epidemic expansion and a pronounced decoupling between the number of detected infections and the risk of hospitalization per infection in several settings. In South Africa, infections identified by an S-gene target failure proxy during the early Omicron wave were associated with lower adjusted odds of hospitalization than contemporaneous non-S-gene target failure infections, although important differences in prior immunity and population characteristics limited interpretation of a purely intrinsic variant effect [20].

Hospital-based comparisons reinforced the observation that disease severity differed between waves. Among 335,219 laboratory-confirmed hospital admissions with known outcomes in a South African surveillance analysis, the proportion experiencing severe disease was 33.6% during the Omicron wave, compared with approximately 52–63% during earlier waves; in-hospital case-fatality also differed materially between periods [21]. Importantly, admission criteria, incidental SARS-CoV-2 detection, accumulated immunity, demographic composition, and evolving clinical management can influence such comparisons. A lower proportion of severe outcomes among recorded infections does not imply that overall health-system pressure must be low, because a very large infection wave can still generate substantial absolute numbers of patients requiring care.

Recent evidence does not support treating all later Omicron descendants as progressively less severe. A 2026 systematic review of 32 studies comparing Omicron subvariants found that hospitalization, intensive care admission, and mortality were broadly comparable across the subvariants assessed, with only limited and often statistically inconclusive evidence of variation [22]. The distinction between intrinsic virulence and observed severity is fundamental: an observed risk ratio combines virological properties with pre-existing immunity, vaccination status, availability of antivirals, changes in testing practice, and the demographic composition of infected patients. Temporal comparisons that do not adjust for these factors are susceptible to confounding and selection bias.

Changes in non-pharmaceutical interventions and behavior also contributed to wave timing and amplitude. A multinational modeling analysis across 131 countries linked the introduction and removal of various non-pharmaceutical interventions to subsequent changes in the time-varying reproduction number, although estimates depended on context and the intervention combinations evaluated [23]. Epidemic growth should be interpreted using transmission indicators alongside counts of severe outcomes. The effective reproduction number is particularly sensitive to the completeness and timing of incidence data, the assumed generation interval, shifts in testing, and the lag between infection and reporting. By 2026, routine reported case curves alone provide a less consistent basis for cross-period comparisons than during periods of broad and stable testing.

Age-, sex-, and population-specific epidemiological patterns

The epidemiological distributions of SARS-CoV-2 infection and severe COVID-19 are distinct. Infection risk reflects contact patterns, occupational exposure, household composition, and immunity, whereas the probability of hospitalization or death following infection is strongly influenced by host factors. Older age has consistently emerged as one of the principal predictors of severe outcomes, with particularly pronounced risk among frail individuals and residents of long-term care facilities. In a population-based English cohort comprising more than 17 million adults, COVID-19-related mortality was associated with a strong age gradient and with male sex, deprivation, obesity-related risk, diabetes, severe asthma, and other chronic conditions [24]. The study was conducted during the early pandemic, and its risk estimates should not be directly transferred to contemporary vaccinated populations without considering changes in absolute risk.

Sex differences have remained epidemiologically relevant, although their magnitude differs by setting and time. In the OpenSAFELY analysis, male sex was associated with a higher risk of

COVID-19-related death (hazard ratio: 1.59; 95% confidence interval (CI): 1.53–1.65) after adjustment for measured covariates [24]. An analysis of age- and sex-specific excess mortality in multiple countries subsequently found that the pandemic amplified existing male–female mortality differences in 2020, particularly in high-income countries, with attenuation of this pattern in 2021 [25]. These findings likely reflect a combination of biological susceptibility, exposure patterns, behavioral factors, comorbidities, and inequitable access to preventive or therapeutic interventions; they do not establish a single mechanism responsible for the observed sex differences.

Children and adolescents generally experienced lower risks of acute fatal outcomes than older adults, but were not epidemiologically unaffected. Pediatric infections contributed to household and school-associated transmission, while severe acute disease remained possible, particularly in infants and children with major underlying conditions. Pregnancy, advanced age, immunosuppressive treatment, and chronic cardiopulmonary or metabolic disorders can alter the risk of clinically important outcomes. In addition, immunocompromised individuals may experience prolonged infection, greater vulnerability to severe disease, and less predictable protection from vaccination. A meaningful risk assessment therefore needs to distinguish the probability of exposure, the probability of infection after exposure, and the probability of progression after infection, rather than treating demographic subgroups as uniformly high- or low-risk.

Social determinants further modify clinical risk. Neighborhood-level research in England found higher COVID-19 mortality in localities characterized by deprivation, population density, poorer background health, and greater representation of employment involving interpersonal contact, with an attenuation of some socioeconomic gradients following vaccine deployment [26]. Such findings are valuable for identifying structurally vulnerable populations but require attention to residual confounding and ecological inference. In clinical and public health practice, prioritization strategies should account for the intersection of older age, comorbidity, immune compromise, occupational exposure, and barriers to prompt diagnosis or treatment.

Risk factors for infection, severe disease, and mortality

Risk factors for acquiring SARS-CoV-2 infection differ fundamentally from determinants of progression to severe COVID-19. Exposure-associated factors include the frequency and duration of close interpersonal contact, indoor crowding, ventilation, occupational circumstances, household infection, and opportunities for respiratory protection. Immunological susceptibility is additionally influenced by elapsed time since vaccination or infection and by the antigenic distance between circulating viruses and previously encountered variants. In contrast, adverse outcomes following infection are more strongly associated with chronological and biological age, frailty, immune suppression, and specific chronic diseases [24]. Failure to separate these causal stages can produce misleading inferences when risk factors are estimated in datasets restricted to those who were tested or hospitalized.

Cardiovascular disease, chronic kidney disease, diabetes, chronic respiratory disease, severe obesity, and immunocompromising conditions have repeatedly been identified as clinically relevant indicators of vulnerability [24]. However, the strength of association is heterogeneous across specific conditions and may change as background vaccination coverage, treatment availability, and the affected population change. Multimorbidity should not be reduced to a binary indicator of any chronic condition because the number, severity, and control of underlying diseases can meaningfully modify absolute risk. Similarly, age-related confounding can distort associations between comorbidity and mortality if not appropriately handled through multivariable modeling, stratification, or standardization.

Outcomes also depend on characteristics of the healthcare pathway. Delayed diagnosis, restricted availability of antiviral treatment, hospital overcrowding, and disruptions in routine management of chronic diseases can influence observed mortality independently of viral biological severity. Selection processes are particularly problematic in studies using hospitalization as both an eligibility criterion and an outcome: admission practices may vary across calendar periods and institutions. Population-based cohorts linked to vaccination, hospitalization, and mortality records generally offer stronger inference than uncontrolled

comparisons of hospitalized patients, although unmeasured confounding remains possible [24,26].

For contemporary epidemiology, reporting should emphasize both relative and absolute measures of risk. A substantial relative risk in a rare outcome may correspond to a small absolute excess risk in a younger population, whereas even a modest relative difference may translate into many deaths among older or highly exposed groups. Estimates should be accompanied by clearly defined outcomes, calendar time, variant context, prior immunity, and denominator ascertainment. Inference about the effectiveness of prevention or treatment additionally requires caution regarding confounding by indication, depletion of susceptible individuals, survivor bias, and differences in healthcare-seeking behavior. The principal epidemiological characteristics and risk factors associated with severe COVID-19, including demographic, clinical, immune, and structural determinants, are summarized in **Table 1**.

Reinfection patterns and changing population susceptibility

Repeated SARS-CoV-2 infection has become a defining feature of the post-emergence epidemiology. As more individuals acquired immunity through vaccination, infection, or both, the frequency of primary infection among immunologically naive persons became a progressively less informative summary of population risk. Omicron evolution substantially modified the durability of protection against subsequent infection. In a population-wide investigation from Qatar, protection conferred by previous infection against reinfection was relatively durable during the pre-Omicron era but declined rapidly during the Omicron era, with little measurable protection remaining approximately one year after an Omicron infection [27]. These estimates were derived from a particular population and variant context, and should not be generalized to every later lineage or population without qualification.

The outcomes prevented by previous infection also differ. A systematic review and meta-analysis of 65 studies found that prior infection conferred considerably lower and more rapidly waning protection against Omicron BA.1 reinfection than against reinfection with pre-Omicron variants, whereas protection against severe disease was more durable across the evaluated variant periods [28]. These results support distinguishing protection against detectable infection, symptomatic infection, hospitalization, and death. A high prevalence of reinfections is therefore compatible with a lower risk of severe disease per episode in immune-experienced populations, without implying that repeated infection is devoid of clinical or long-term consequences.

In a 2025 systematic review and meta-analysis including 36 studies, the pooled cumulative incidence of documented reinfection during the Omicron period was 3.35% (95%CI: 1.95–5.72%). The pooled incidence was higher among healthcare workers and working-age adults than among the general population, although the contributing studies differed considerably in follow-up, reinfection definitions, testing access, and exposure [29]. This pooled measure should not be interpreted as the true lifetime probability of reinfection: episodes detected by routine surveillance generally miss asymptomatic or untested infections, and a cumulative incidence estimate is strongly determined by the observation period. The same review identified limited and heterogeneous evidence regarding hospitalization and long-term outcomes after reinfection [29].

Population susceptibility is now heterogeneous and dynamic. Protection is shaped by time since the most recent antigenic exposure, prior variant history, vaccine formulation, immune competence, and ongoing viral evolution. Reinfection may also be misclassified when prolonged RNA detection is not adequately distinguished from a new infection. Studies based on sequencing-confirmed reinfection or well-defined longitudinal testing offer more specific evidence but are less widely available. As a result, forecasts based on a single homogeneous susceptibility parameter are unlikely to represent the full complexity of contemporary SARS-CoV-2 transmission.

Table 1. Epidemiological characteristics and risk factors associated with severe COVID-19

Characteristic or exposure	Epidemiological evidence and observed association	Interpretation and important limitations	References
Older age	The risks of hospitalization, organ failure, and death rise markedly with age, with the steepest absolute burden concentrated among older adults.	Age effects persist after accounting for major comorbidities; frailty, immune aging, and care-setting exposure further modify risk.	[24, 117, 143]
Male sex	Male patients have shown higher rates of intensive care admission and death than female patients in pooled cohorts.	Biological, behavioral, comorbidity, and healthcare-access pathways may contribute; effects vary by population and variant era.	[24, 144]
Obesity	Higher body mass index, particularly severe obesity, is associated with hospitalization and adverse respiratory outcomes.	May involve reduced respiratory reserve, metabolic dysfunction, and inflammatory alterations; avoid interpreting association as solely causal.	[24, 142]
Diabetes mellitus	Pre-existing diabetes is associated with higher risk of severe disease and mortality in multiple observational cohorts.	Glycaemic control, coexisting obesity, age, and cardiovascular disease affect estimates; treatment exposures are potential confounders.	[24, 118, 119]
Cardiovascular disease and hypertension	Established cardiovascular disease is consistently linked to poor outcomes; hypertension often co-occurs in hospitalized populations.	Cardiovascular disease has stronger prognostic specificity than isolated hypertension after adjustment for age and multimorbidity.	[24, 119, 123]
Chronic kidney disease	Chronic kidney disease is a major predictor of hospitalization and COVID-19-related death.	Risk generally increases with disease severity; dialysis, transplant status, and impaired immune response create distinct vulnerabilities.	[24, 127, 143]
Chronic lung disease	Chronic obstructive pulmonary disease and compromised baseline respiratory function are associated with adverse outcomes.	Underlying diagnosis, disease severity, smoking history, and oxygen dependence should be evaluated separately.	[24, 143]
Cancer and immunosuppression	Active malignancy, organ transplantation, and other states of impaired immunity may confer higher risks of progression or prolonged infection.	Risk differs greatly by malignancy, therapy, B-cell depletion, immune status, and timing since treatment.	[136, 137, 192]
Pregnancy	Compared with nonpregnant reproductive-age women, pregnant women with symptomatic infection can face greater risk of intensive care treatment and respiratory support.	Absolute risk, trimester, obstetric comorbidity, variant, and vaccination status influence interpretation; fetal and maternal outcomes require separate reporting.	[135, 243]
Children and adolescents	Most pediatric infections are mild, but infants and children with medical complexity can experience severe disease; MIS-C is a recognized uncommon postinfectious complication.	Age-specific hospitalization and severe-outcome definitions are essential; early-pandemic pediatric rates should not be assumed for current variants.	[130, 132, 133]
Previous infection and reinfection	Prior infection is associated with protection against reinfection and, more durably, against severe outcomes; protection against infection changes with antigenic evolution.	Cumulative reinfections may still contribute to acute and postacute burden; immune history is difficult to measure in surveillance data.	[27–29, 208]
Vaccination and hybrid immunity	Vaccination and hybrid immunity reduce risk of severe outcomes; contemporary vaccine effectiveness varies by time since receipt, lineage, and host risk.	Estimates depend on prior exposure, comparator group, endpoint, age, and calendar time; avoid equating protection against infection with protection against death.	[9, 112, 240]
Variant period and population immunity	Observed severity differed between Delta and Omicron waves, with lower average per-infection severity reported for early Omicron in several settings.	Differences reflect both intrinsic viral traits and changing immunity, testing, admission thresholds, and treatment availability.	[20–22, 138, 139]
Socioeconomic and structural determinants	Deprivation, occupational exposure, household crowding, and unequal access to care shape infection and outcome disparities.	Associations are context-dependent; ethnicity or geography should not be treated as intrinsic biological causes.	[24–26, 249]

ICU: intensive care unit; MIS-C: multisystem inflammatory syndrome in children

Epidemiological characteristics in low- and middle-income countries

Low- and middle-income countries (LMICs) have experienced COVID-19 under epidemiological and health-system conditions that differ substantially from those of higher-income settings. Younger population age structures may partially mitigate population-wide mortality rates, whereas household crowding, informal employment, limited social protection, chronic disease burden, uneven access to diagnostics, and variable availability of oxygen therapy or intensive care can increase vulnerability. Accordingly, crude cross-country comparisons of notified infections, deaths, or case-fatality ratios are often inappropriate. Disparities in civil registration and surveillance intensity further complicate assessment of whether observed between-country differences are genuine or primarily reflect the measurement system [4,15].

A central problem is that the absence of detected disease does not establish the absence of SARS-CoV-2 circulation. Representative seroprevalence studies demonstrated that infection exposure frequently exceeded the number of officially notified cases, with especially large discrepancies in settings with restricted access to testing [14,18]. However, the interpretation of serological data has become more complex following vaccination because assays directed against spike protein cannot generally distinguish vaccine-induced from infection-induced seropositivity. Nucleocapsid-based assays can improve attribution to infection in some contexts, but waning antibodies, assay performance, and variation in vaccine platforms limit direct comparability.

Global analyses illustrate the importance of assessing total rather than exclusively reported mortality. The GBD 2020–2021 excess-mortality study estimated a substantial burden in South Asia, including an estimated 736,000 excess deaths in Indonesia (95%UI: 594,000–955,000) over the two-year period [4]. This modeled estimate reflects the total mortality impact associated with the pandemic rather than a verified count of deaths directly caused by SARS-CoV-2. It should therefore not be substituted for national certified COVID-19 mortality in clinical descriptions. Differences between modeled and reported outcomes warrant examination of data quality, access to healthcare, and indirect pandemic effects, rather than simplistic comparisons of national performance.

Vaccination inequity was particularly consequential for LMICs during the acute pandemic period. The global vaccine-impact model estimated that large numbers of deaths were prevented after vaccine introduction, but that additional mortality could have been averted if coverage targets had been achieved in countries with constrained supply [19]. Implementation disparities persisted beyond procurement, encompassing the availability of trained personnel, reliable distribution systems, equitable access for remote communities, and public trust. In the post-emergency period, sustainable COVID-19 surveillance and targeted preventive services should be incorporated into existing respiratory disease and immunization programs rather than depending on parallel short-term emergency infrastructure.

Current epidemiological surveillance and limitations of reported data

The transition away from universal emergency reporting has substantially changed the evidentiary basis of COVID-19 surveillance. Passive notification of laboratory-confirmed cases is susceptible to variable healthcare-seeking behavior, differential test availability, use of unreported home antigen testing, changes in test reimbursement, and the removal of mandatory case notification. Consequently, falling case counts cannot, in isolation, establish declining infection incidence. Likewise, crude case-fatality ratios are especially vulnerable to changes in the denominator and to the lag between diagnosis and death. Analyses intended to compare variant periods or countries should therefore specify surveillance definitions and assess the stability of ascertainment mechanisms.

Integrated surveillance approaches can partly mitigate these weaknesses. Sentinel surveillance for influenza-like illness and severe acute respiratory infection offers a framework for monitoring the proportion of respiratory disease associated with SARS-CoV-2 while simultaneously evaluating influenza and other respiratory pathogens. Linkage to hospital admission records, intensive care utilization, oxygen requirements, and vital registration provides complementary measures of clinical burden. Nevertheless, test positivity cannot directly be treated as population prevalence when sampling is nonrandom, and admission counts require distinction between hospitalization because of COVID-19 and incidental detection of SARS-CoV-2 among patients admitted for other indications.

Wastewater-based epidemiology has emerged as a complementary indicator of community viral circulation. A 2024 survey reviewed the use of SARS-CoV-2 RNA measurements in wastewater and highlighted their potential to track changes in community transmission independently of individual clinical test uptake, while also identifying difficulties in sampling design, laboratory standardization, analytical interpretation, and epidemiological modeling [30]. Interpretation must account for variations in sewer coverage, population mobility, wastewater dilution, viral shedding, and laboratory recovery efficiency. Wastewater concentrations are generally more informative for changes within a stable catchment over time than for deriving a precise number of incident infections or comparing absolute prevalence across different catchments.

The translation of environmental signals into action is another major limitation. A 2025 scoping review of 974 wastewater surveillance studies identified only 84 studies that described public health actions, indicating that the generation of surveillance data does not automatically lead to operational decisions [31]. Effective surveillance consequently requires predefined thresholds or decision frameworks, coordination between laboratories and health authorities, and explicit evaluation of whether surveillance findings improve prevention, treatment access, or resource allocation. Genomic sequencing of clinical and environmental specimens can additionally detect changes in the composition of circulating lineages, but genomic surveillance is also vulnerable to sampling bias and delays between specimen collection and sequence availability.

For the remainder of the decade, reliable interpretation of COVID-19 epidemiology will depend on transparent triangulation of multiple imperfect data streams. Estimates should specify the sampling population, time interval, clinical endpoint, measurement method, and uncertainty; account for changes in vaccination and prior infection; and avoid presenting sparse surveillance data as a precise national infection burden. Particular priority should be given to sustained monitoring of severe outcomes in older and immunocompromised populations, geographically representative sentinel networks, and the strengthening of civil registration systems in resource-constrained settings. Such an approach is essential for distinguishing changes in actual disease burden from changes in the probability that infections or deaths are observed.

Virology and genomic evolution of SARS-CoV-2

Taxonomy and classification of SARS-CoV-2

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus assigned to the species *Betacoronavirus pandemicum* within the subgenus *Sarbecovirus*, genus *Betacoronavirus* and family *Coronaviridae*. Its phylogenetic relationship to SARS-related coronaviruses circulating in mammals situates the virus within a wider reservoir of sarbecovirus diversity. Comparative genomic analyses demonstrated substantial divergence of the SARS-CoV-2 lineage from sampled close relatives over evolutionary timescales, with recombination complicating the reconstruction of deep ancestry [32]. Viral taxonomy must nevertheless be distinguished from the classification of contemporary epidemic lineages, which describes variation among SARS-CoV-2 genomes circulating in human populations and does not assign each lineage to a separate species.

The approximately 30-kilobase coronavirus genome combines the rapid evolutionary potential of RNA replication with a larger coding capacity than that of most other known RNA viruses. This genomic architecture is supported by a replication machinery that includes RNA-dependent RNA polymerase activity, helicase functions, RNA-capping enzymes and a proofreading exonuclease. Conserved aspects of the coronavirus life cycle provide a mechanistic framework for understanding SARS-CoV-2 entry, intracellular replication, virion assembly and transmission [33]. At the same time, relatively modest sequence changes in defined functional regions can produce substantial phenotypic differences, particularly where host receptor recognition or antibody binding is affected.

Classification of SARS-CoV-2 therefore operates at several complementary levels. Taxonomic designations characterize deep evolutionary relationships; phylogenetic lineage names capture transmission-relevant diversification; and public health categories group variants according to evidence of potential or demonstrated epidemiological consequences. The term

'variant' does not, by itself, establish higher transmissibility, severity or immune escape. Such properties require separate evidence from sequence epidemiology, structural experiments, neutralization studies, epidemiological investigations and clinical outcomes [7,32,33]. These distinctions are essential when interpreting the increasingly complex nomenclature of Omicron descendants.

Viral structure and genomic organization

The SARS-CoV-2 genome consists of positive-sense RNA with a 5′ cap and 3′ polyadenylated terminus. The 5′ two-thirds of the genome contain overlapping open reading frames ORF1a and ORF1b; programmed −1 ribosomal frameshifting permits translation of pp1a and the longer pp1ab polyprotein (**Figure 1**). Viral proteolytic processing generates nonstructural proteins (nsps) involved in RNA synthesis, host-cell remodeling and modulation of antiviral responses. The 3′ genomic region encodes four principal structural proteins—spike (S), envelope (E), membrane (M) and nucleocapsid (N)—as well as lineage-dependent accessory proteins expressed predominantly through subgenomic messenger RNAs [34]. Changes in accessory open reading frames may influence viral-host interactions, although phenotypic effects remain context-dependent.

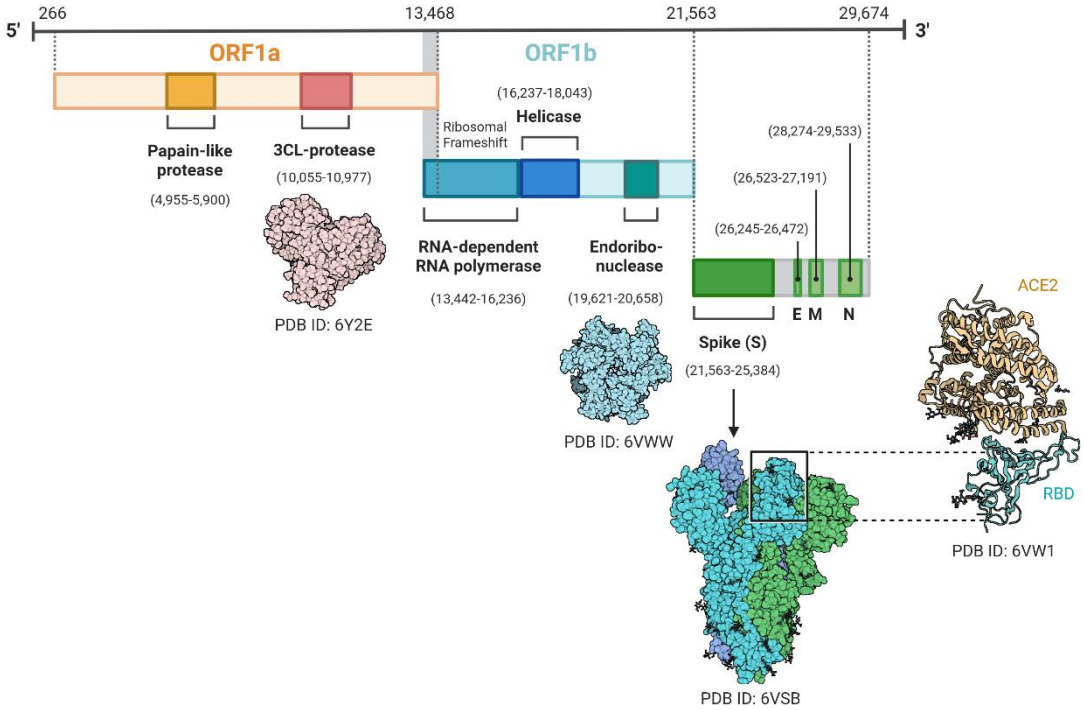


Figure 1. Genomic organization of SARS-CoV-2 and key viral proteins. The positive-sense RNA genome contains ORF1a and ORF1b, which encode polyproteins processed into nonstructural proteins involved in viral replication. The 3′ region encodes the structural proteins spike (S), envelope (E), membrane (M), and nucleocapsid (N). Representative protein structures illustrate the viral proteases, RNA-dependent RNA polymerase, spike protein, and interaction between the spike receptor-binding domain (RBD) and human angiotensin-converting enzyme 2 (ACE2).

Virions contain a ribonucleoprotein complex formed by genomic RNA and N protein, enclosed within a lipid envelope incorporating S, M and E. S is a trimeric class I fusion glycoprotein and consists of a receptor-binding S1 region and a fusion-mediating S2 region. Cryogenic electron microscopy of the prefusion trimer revealed conformational heterogeneity in its receptor-binding domains (RBDs), including receptor-accessible 'up' conformations; these observations provided a structural foundation for vaccine antigen design and the study of antibody neutralization [35]. Membrane protein is central to particle morphology and assembly; E contributes to assembly and membrane dynamics, and N supports RNA packaging while engaging multiple cellular processes [33,34].

Attachment is mediated primarily through the interaction of the S RBD with host angiotensin-converting enzyme 2 (ACE2). Structural studies of SARS-CoV-2 S bound to human ACE2 identified the receptor-contact interface and amino acid interactions underlying receptor recognition [36]. Binding affinity, however, represents only one component of entry competence: the proportion of spike in an accessible conformation, cleavage status, glycan distribution, cellular protease availability and membrane-fusion kinetics also contribute to infection efficiency. Changes at the RBD and its surrounding antigenic surfaces can influence receptor engagement and antibody accessibility simultaneously, creating evolutionary trade-offs that cannot be inferred from a single mutation.

Productive entry additionally requires proteolytic priming. Host proteases, including transmembrane serine protease 2 (TMPRSS2), can facilitate cell-surface fusion, whereas endosomal cathepsins support entry through an alternative route in appropriate cellular contexts. The requirement for ACE2 and the contribution of TMPRSS2 were established in foundational cell-entry experiments [37]. These pathways vary with target-cell phenotype and viral genotype and should not be considered invariant properties of all SARS-CoV-2 lineages. The furin-cleavage site at the S1/S2 boundary is an important determinant of spike processing and can modify the balance between plasma-membrane and endosomal entry.

The metastability of prefusion S provided an additional translational insight. Structure-guided substitution strategies produced more stable recombinant antigens, including the HexaPro construct, which improved protein expression and stability while preserving prefusion architecture [38]. This advance illustrates how structural virology has directly informed vaccine engineering. Nevertheless, spike-focused interventions exert selective pressure on antigenic epitopes, and sequence diversification outside spike remains relevant to viral replication and the response to direct-acting antivirals.

Structural, nonstructural and accessory viral proteins

The biological functions of the four major structural proteins are interdependent rather than isolated. S initiates receptor engagement and fusion, whereas M acts as an organizing component of virion morphogenesis. E is present in comparatively small quantities in mature particles and contributes to membrane curvature and assembly. N binds genomic RNA through multiple interaction domains and supports ribonucleoprotein condensation, genome incorporation and coordination between replication and assembly [33,34]. Because N is generally more conserved than the exposed S antigenic regions, it has been extensively investigated as a diagnostic antigen, although changes in N sequence can still affect specific immunoassays.

The nonstructural proteins form a multifunctional apparatus that supports the unusually complex coronavirus replication cycle. Nonstructural proteins 3 and 5 contain the papain-like and main protease activities responsible for pp1a/pp1ab processing. Nonstructural protein 12 is the catalytic RNA-dependent RNA polymerase, assisted by cofactors including nsp7 and nsp8. Nonstructural protein 13 has helicase activity, whereas nsp14 contributes proofreading exonuclease and methyltransferase functions; other enzymes support RNA-cap maturation, membrane rearrangement and antagonism of host defenses [33,34]. This division of labor also identifies multiple antiviral targets, although conservation and druggability vary across enzymes.

Accessory proteins, including ORF3a, ORF6, ORF7a and ORF8, are neither uniform in evolutionary stability nor dispensable for all aspects of viral biology. They participate in host-response modulation in experimental systems, including interference with interferon signaling, antigen processing and intracellular trafficking. Mutations, deletions and changes in accessory-gene expression have been documented across lineages, but an isolated alteration cannot be assumed to produce a reproducible change in clinical severity. Its effect may depend on the broader genomic background, interacting viral proteins and the immune competence of the host [7,34]. Accordingly, functional characterization should extend beyond spike when evaluating substantially diverged lineages.

Protein-level interpretation further requires attention to epistasis. The phenotypic effect of an individual substitution may depend on the presence of other substitutions in the same protein or elsewhere in the genome. Mutations that improve antibody evasion may compromise folding or receptor binding and subsequently be compensated by changes in other regions. Such relationships help explain why genetically related lineages can display different growth

advantages and why the evolutionary consequences of specific substitutions are not necessarily stable across successive variant backgrounds [6,7].

SARS-CoV-2 entry, replication, transcription and assembly

Following S-mediated fusion or endosomal entry, genomic RNA is released into the cytoplasm and functions directly as messenger RNA. Translation of ORF1a/ORF1b produces pp1a and pp1ab, which are cleaved to generate the replication–transcription complex. Coronavirus infection induces extensive remodeling of endoplasmic-reticulum-derived membranes, producing double-membrane vesicles (DMVs) that provide specialized sites for viral RNA synthesis (**Figure 2**) [33,34]. The organization of these replication compartments is important for efficient production of viral RNA and for interactions between viral RNA intermediates and innate immune sensing pathways.

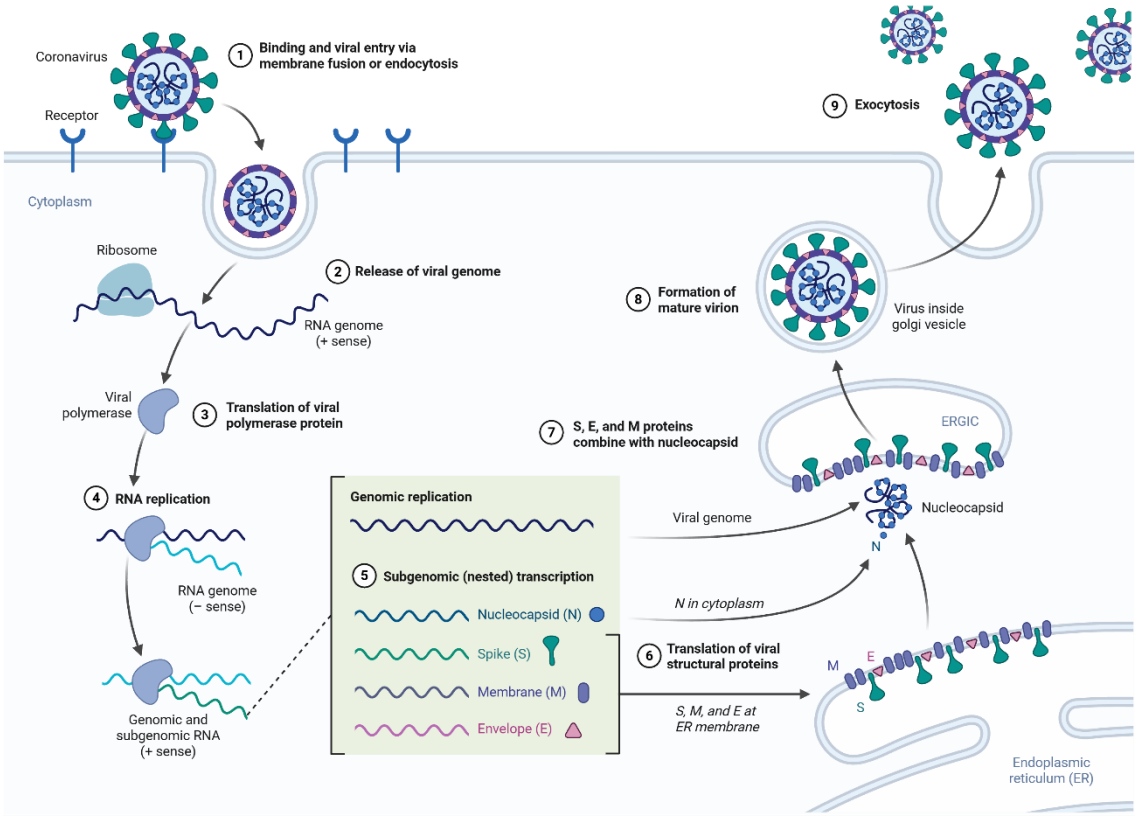


Figure 2. Coronavirus replication cycle. Following viral entry and genomic RNA release, translation and proteolytic processing of viral polyproteins generate nonstructural proteins that form the replication–transcription complex. RNA synthesis occurs in endoplasmic-reticulum-derived double-membrane vesicles (DMVs), followed by structural protein synthesis, virion assembly at the endoplasmic reticulum–Golgi intermediate compartment (ERGIC), and release by exocytosis. S, spike; E, envelope; M, membrane; N, nucleocapsid.

The existence of membrane-enclosed replication compartments raised a fundamental transport problem: newly synthesized viral RNA must exit the DMV for translation or incorporation into virions. Cryogenic electron microscopy first identified a pore-like complex spanning the DMV membrane [39]. Subsequent structural characterization resolved the architecture of a pore assembled from nsp3 and nsp4, providing a molecular explanation for the transport pathway that connects RNA synthesis within the vesicle with its functions in the surrounding cytoplasm [40]. These findings have clarified coronavirus replication organelle biogenesis and suggest potential vulnerabilities distinct from the viral polymerase active site.

Genome replication proceeds through complementary negative-sense RNA intermediates, whereas discontinuous transcription generates a nested set of subgenomic RNAs through interactions between transcription-regulatory sequences. These RNAs support differential expression of downstream structural and accessory genes [34]. The abundance of individual transcripts varies by transcription-regulatory signals, RNA stability and infection stage; therefore, detecting a specific subgenomic RNA in a clinical specimen cannot automatically be

interpreted as proof of the presence of replication-competent virus. The biological meaning of RNA detection must be evaluated alongside specimen type, infection timing and, where appropriate, viral culture or other complementary data.

Structural proteins are synthesized and trafficked through host-cell membranes, with assembly occurring predominantly at the endoplasmic reticulum–Golgi intermediate compartment. Genomic RNA associated with N is packaged into budding particles, followed by maturation and release through cellular secretory pathways [33]. Each step is potentially influenced by host-cell state and viral genotype. Experimental observations in immortalized cell lines, primary airway epithelium, organoids and animal models are thus valuable but not interchangeable; cell-specific receptor and protease expression, innate immunity and drug metabolism can markedly influence the resulting phenotypes.

Molecular mechanisms of mutation, recombination and selection

SARS-CoV-2 evolution reflects a combination of replication-associated substitutions, insertions and deletions, RNA recombination and selection acting across viral populations. Unlike many RNA viruses, coronaviruses encode an nsp14 exonuclease that improves replication fidelity, thereby supporting the maintenance of a relatively large RNA genome. Proofreading does not prevent evolutionary diversification: substitutions affecting nsp14 itself may alter the distribution and accumulation of mutations. Experimental investigations demonstrated that the nsp14 P203L change was associated with greater genomic diversity during viral replication [41]. More recent reverse-genetics work showed that loss of nsp14 exonuclease activity impairs SARS-CoV-2 replication, fidelity, and fitness, indicating that proofreading is simultaneously a constraint on mutation and a requirement for efficient viral propagation [42].

Not all observed substitutions reflect adaptation. Genetic drift, founder effects, changes in sampling, and the linkage of neutral mutations to advantageous variants can produce pronounced changes in lineage frequency. Evidence for positive selection is strengthened when phylogenetic growth is replicated across settings and accompanied by laboratory findings consistent with higher fitness. The early rise of spike D614G illustrates the value of this combined approach: epidemiological and experimental analyses identified an infectivity-associated phenotype, without establishing that the substitution independently caused more severe clinical disease [43]. The distinction between greater viral fitness and greater virulence remains fundamental.

Recombination provides an additional source of genomic novelty. During coinfection, the coronavirus polymerase may switch templates, producing genomes containing segments from different parental lineages. Such events can combine antigenic or functional changes that arose independently, potentially allowing rapid alterations in spike composition or replication properties. However, inference of recombination requires careful exclusion of sequencing contamination, mixed infections and phylogenetic artifacts. A recombinant designation does not, without phenotypic evidence, imply enhanced transmission or disease severity [7,33].

The growing complexity of SARS-CoV-2 diversity motivated a dynamic lineage classification system that links phylogenetic structure with epidemiological relevance while preserving a tractable naming scheme [44]. Lineage names represent inferred relationships among sampled genomes, not an unambiguous record of the location or mechanism of origin. Short evolutionary branches may be densely sampled, whereas long unsampled branches can reflect geographic surveillance gaps, prolonged infection or sustained replication in poorly characterized transmission networks. Selection also depends on the immune landscape: as population immunity accumulates, mutations conferring escape from prevailing antibodies may provide an advantage even when their intrinsic replicative benefits are limited.

Persistent infection provides a biologically plausible setting for accelerated within-host diversification, particularly where viral clearance is impaired. Nevertheless, identifying an unusual evolutionary branch or mutations previously observed in chronically infected patients does not establish that a given epidemic lineage arose from an immunocompromised host. Alternative explanations include unsampled transmission chains and recombination. Interpretation should integrate longitudinal viral sequences, host clinical information where available, population phylogenetics and functional studies, while avoiding causal attribution beyond the available evidence [7].

Emergence and virological characteristics of major variants

The early global spread of SARS-CoV-2 was followed by sequential expansion of genetically distinct lineages with differing epidemiological advantages. D614G provided an early example of a spike substitution associated with improved infectivity; subsequently, Alpha, Beta, Gamma and Delta displayed differing combinations of receptor-binding changes, altered susceptibility to neutralizing antibodies and replication phenotypes [7,43]. Delta became associated with a substantial burden of severe disease in populations with limited protection, but comparisons between successive waves are influenced by vaccination coverage, prior infection, age structure, treatment access and case ascertainment. Therefore, intrinsic pathogenicity cannot be established from ecological differences in hospitalization or mortality alone.

The emergence of Omicron in late 2021 represented a major antigenic and functional shift. Omicron BA.1 carried numerous substitutions in spike, several affecting antibody epitopes or receptor engagement, and showed considerable resistance to neutralization by sera elicited by early vaccine formulations. Importantly, this viral change was not confined to antibody escape. Experiments demonstrated altered spike processing, reduced dependence on TMPRSS2-mediated fusion in selected cell types, and greater utilization of endosomal entry compared with Delta [45]. These findings supplied a mechanistic explanation for differences in cellular tropism, although extrapolation from cell-entry assays to individual clinical outcomes requires considerable caution.

Independent investigations supported the distinction between Omicron antigenic properties and cell-entry biology. BA.1 and BA.2 showed substantial reductions in neutralization by vaccine-induced antibodies in vitro, together with differences in syncytium formation and a preference for endosomal entry in the tested systems [46]. Such observations do not support a simple classification of Omicron as uniformly attenuated across all tissues, hosts or subsequent descendant lineages. Clinical severity is the outcome of interacting viral and host factors, and comparisons require adjustment for prior immunity and evolving clinical practices.

Variant replacement can reflect multiple nonexclusive mechanisms. Higher receptor affinity may improve entry under certain conditions, changes in spike stability or protease usage may alter cell-type compatibility, and immune escape may increase the proportion of susceptible hosts in a previously exposed population. Epidemiological expansion may also be influenced by founder effects, behavior, and population mobility. Moreover, a lineage that is advantageous in a specific immune environment may not remain so as the immune landscape changes. A comprehensive interpretation of major variant transitions therefore requires convergence of genomic, laboratory and population-based evidence rather than reliance on any one metric [6,7].

Evolution of Omicron descendants and contemporary variants

Following the initial Omicron waves, viral diversification increasingly occurred within Omicron-derived genetic backgrounds. BA.2, BA.5 and subsequent XBB descendants acquired distinct constellations of substitutions that modified the antibody-recognition landscape. More recently, BA.2.86 and its JN.1 descendant marked a major evolutionary departure from contemporary XBB lineages. Experimental comparisons of XBB and BA.2.86/JN.1 variants demonstrated different combinations of viral replication, upper-airway cell infection, ACE2 interaction and antibody evasion [47]. Such data emphasize that progressive antigenic drift need not produce a uniform directional change in all measures of viral fitness.

The transition from XBB to JN.1 illustrates how preexisting humoral immunity shapes selection. Detailed mapping of antibody repertoires and viral neutralization demonstrated antigenic differences between lineages, while JN.1 descendants such as KP.2 and KP.3 acquired additional escape properties affecting subsets of neutralizing antibodies [48]. Nevertheless, neutralization titers are surrogate laboratory outcomes whose translation into infection risk depends on immune history, time since exposure, mucosal immunity, and other host characteristics. Protection against hospitalization and death can persist despite reduced neutralization against infection, particularly when cellular and nonneutralizing immune mechanisms remain active.

Spike substitutions are not the sole determinants of lineage replacement. Reverse-genetics experiments comparing BA.2.86 with JN.1 found that changes in non-spike proteins, including nsp6 and ORF7b, could compensate for a fitness cost associated with a JN.1 spike substitution

[49]. These observations provide mechanistic evidence for epistasis across the viral genome and challenge interpretations of evolutionary success based exclusively on RBD affinity or antigenic maps. They also reinforce the importance of whole-genome sequencing (WGS) rather than spike-only analysis when evaluating newly expanding lineages.

During 2025, lineages including LP.8.1, NB.1.8.1 and XFG attracted attention because of distinct spike substitutions and antigenic properties. Initial analyses of NB.1.8.1 provided virological characterization of this lineage, while subsequent antibody and receptor-binding studies identified differences between NB.1.8.1, XFG, LP.8.1-related viruses and preceding Omicron descendants [50,51]. In one comparative study, NB.1.8.1 and XFG were more resistant to serum neutralization than LP.8.1.1 in the tested cohort, suggesting that antibody escape contributed to their emergence [51]. These results are laboratory comparisons and, on their own, do not demonstrate greater clinical severity or a stable global predominance during 2026.

The identification and study of BA.3.2 further illustrate the possibility of antigenically divergent lineages reappearing from long, incompletely sampled branches. Experiments with BA.3.2 spike constructs demonstrated pronounced neutralization escape but reduced entry and cell–cell fusion relative to the corresponding comparator viruses; alterations in glycosylation contributed to the observed phenotype [52]. Because much of this evidence is derived from pseudovirus and cellular assays, conclusions about real-world transmissibility require additional epidemiological data. Laboratory fitness can be context-dependent, and antigenic divergence alone does not establish epidemic dominance.

Comparative investigations published in 2026 have also evaluated LP.8.1.1, XEC.25.1, XFG and NB.1.8.1, describing differences in cell entry, ACE2 engagement, fusogenicity and serum neutralization [53]. The coexistence of enhanced receptor binding in one lineage and higher cell–cell fusion in another illustrates the multidimensional nature of viral adaptation. It would therefore be inappropriate to rank contemporary variants on a single biological scale of 'more infectious' without specifying the assay, host-cell substrate and epidemiological outcome. As of October 2026, the list and proportions of circulating lineages are inherently time sensitive and should be updated using contemporaneous sequence surveillance when the review is submitted. The major SARS-CoV-2 lineages and their virological, antigenic, and clinical characteristics are summarized in **Table 2**.

Implications of viral evolution for transmissibility, immune escape and pathogenicity

Viral evolution affects transmission through mechanisms operating at multiple biological scales. Receptor recognition and proteolytic activation influence susceptibility of individual cells, replication kinetics and shedding affect opportunities for onward infection, and antigenic escape modifies the fraction of hosts who are susceptible to reinfection. These factors interact with nonvirological determinants, including behavior, exposure intensity and prior immunity. Consequently, a faster increase in the proportion of a lineage does not uniquely identify the biological mechanism driving its growth. Epidemiological studies should distinguish changes in intrinsic viral properties from changes in the immune and behavioral environment [7,45,47].

Neutralizing-antibody escape is especially important for interpreting repeated waves in highly exposed populations. Substitutions in the receptor-binding domain and N-terminal domain can disrupt antibody binding, alter local glycosylation or shift spike conformation. Such effects may be selective for particular antibody classes rather than uniform across the polyclonal response. Furthermore, the serum neutralization phenotype depends on the cohort studied: antibody repertoires vary after ancestral vaccination, variant-adapted vaccination, infection with different lineages and hybrid exposure histories. Antigenic distance should therefore be assessed across well-characterized immune histories and not treated as an invariant property of the virus [48,51,52].

Pathogenicity is more difficult to infer than antibody escape. Mechanistic changes in tropism, fusion or inflammatory signaling may modify virulence in experimental models, yet clinical outcomes are strongly influenced by the age and comorbidity of infected individuals, vaccination history, access to antivirals, and the proportion of incidental infections identified during hospital screening. Apparent reductions in severity between early and later pandemic periods cannot be assigned solely to viral attenuation.

Table 2. Major SARS-CoV-2 variants and their virological, immunological, and clinical characteristics

Variant/lineage	Virological and evolutionary features	Antigenic and immune characteristics	Epidemiological/clinical interpretation	References
Ancestral lineages (2019–2020)	Wuhan-like SARS-CoV-2; ACE2-dependent entry supported by spike activation and host proteases.	Baseline antigenic reference for first-generation vaccines and neutralization studies.	Early-pandemic case fatality and hospitalization estimates were strongly influenced by limited testing and largely immunologically naive populations.	[1, 35–37]
D614G-bearing lineages	Spike D614G became globally widespread and was associated with enhanced infectivity/transmission fitness in experimental and epidemiological analyses.	Limited antigenic divergence relative to later variants; not equivalent to a distinct clinical severity phenotype.	Displacement of ancestral lineages demonstrated rapid adaptive evolution.	[7, 43]
Alpha (B.1.1.7)	Spike mutations included N501Y and P681H; increased fitness relative to earlier circulating lineages.	Some reduction in neutralization for selected sera, less marked than with Beta or Omicron.	Rapid expansion in 2020–2021; observed clinical risk depends on study design and epidemic context.	[7, 44]
Beta (B.1.351) and Gamma (P.1)	Independent evolutionary lineages with immune-relevant receptor-binding-domain substitutions, including E484K and N501Y.	Substantial resistance to subsets of infection- or vaccine-elicited neutralizing antibodies.	Important examples of convergent antigenic adaptation; historical, rather than dominant contemporary, lineages.	[7]
Delta (B.1.617.2/AY)	Efficient airway replication, spike-mediated entry and high transmission fitness during its major circulation period.	Partial reduction in neutralization relative to ancestral viruses, but less antigenically distant than Omicron.	Associated with substantial severe disease burden; comparisons with Omicron require adjustment for host immunity and age.	[7, 138, 139]
Omicron BA.1 and BA.2	Large spike substitution burden, altered cellular entry pathways, and reduced TMPRSS2 dependence relative to Delta in experimental models.	Pronounced escape from many ancestral-strain neutralizing antibody responses.	Marked transmission in populations with previous immunity; lower observed severity than Delta in several cohorts, not absence of severe disease.	[20, 45, 46]
Omicron BA.4/BA.5	Further spike evolution improved fitness in immune-experienced populations.	Additional neutralization escape compared with earlier Omicron sublineages.	Recurrent infection waves demonstrated the limits of infection-blocking immunity.	[7, 140]
XBB family	Recombinant Omicron descendants exhibiting convergent evolution of receptor binding and antibody evasion.	Marked antigenic divergence from ancestral vaccine strains; motivated variant-updated vaccine evaluation.	Clinical severity cannot be inferred directly from antibody neutralization assays.	[7, 47, 238]
BA.2.86 and JN.1	A highly diverged BA.2-derived background; subsequent JN.1 evolution involved both spike and non-spike determinants of viral fitness.	Antigenic shift and immune escape relative to XBB-era responses; neutralization depends on recent immune history.	Substantial population spread despite heterogeneous clinical outcomes across settings.	[47–49]
JN.1 descendants: KP.3, XEC, LP.8.1	Successive substitutions and lineage competition altered receptor binding and antigenicity during 2024–2025.	Variant-specific neutralization patterns and waning immunity affect vaccine effectiveness estimates.	Continued antigenic turnover supports recurrent vaccine-strain assessment and genomic monitoring.	[51, 56, 240]
NB.1.8.1	Omicron descendant with experimentally characterized entry and neutralization properties.	Serum and monoclonal antibody escape differs from LP.8.1 and other contemporaneous descendants.	Growth advantage does not itself demonstrate higher intrinsic virulence.	[50, 51]
XFG	Recombinant Omicron lineage with distinctive spike antigenicity in laboratory comparisons.	Increased neutralization escape compared with some LP.8.1 descendants in tested sera.	Population-level severity and transmissibility must be assessed from clinical and surveillance data, not binding assays alone.	[51]
BA.3.2	Highly divergent evolutionary trajectory from earlier Omicron BA.3; altered spike glycosylation, cell entry, and fusion in experimental systems.	Marked neutralization resistance; laboratory experiments suggest trade-offs between entry efficiency and immune escape.	Clinical consequences remain incompletely established; laboratory findings should not be extrapolated directly to patient outcomes.	[52]

ACE2: angiotensin-converting enzyme 2; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; TMPRSS2: transmembrane serine protease 2

Conversely, increased immune escape does not necessarily entail greater intrinsic virulence. Experimental comparisons should specify cell type, viral inoculum and animal model, and population-based studies should address confounding and differences in outcome ascertainment [20,21,45].

Evolution also has implications for the durability of medical countermeasures. Spike changes can reduce susceptibility to individual monoclonal antibodies and change the match between circulating viruses and vaccine immunogens, whereas substitutions in non-spike targets may influence direct-acting antiviral susceptibility. Ongoing evaluation should therefore integrate phenotypic antiviral assays, clinically relevant resistance surveillance, and genomic monitoring. A mutation predicted to reduce drug binding does not establish clinically important resistance without corroborating experimental and, where available, clinical evidence. The changing virus population argues for broadly active therapeutic targets, diversified antigenic coverage, and timely updating of preventive interventions [6,7].

Persistent infection in some immunocompromised individuals represents a related but distinct clinical and evolutionary concern. Prolonged replication under incomplete immune pressure may permit within-host accumulation of mutations and provide opportunities for selection or recombination. A review of such infections described mutations overlapping with those subsequently found in variants of concern, but emphasized uncertainty about the origins of particular epidemic variants [54]. This distinction is scientifically and ethically important: immunocompromised individuals should not be presented as the demonstrated source of a specific lineage in the absence of direct phylogenetic and epidemiological evidence.

Genomic surveillance, sequencing technologies and emerging challenges

Genomic surveillance translates viral sequence variation into evidence about lineage emergence, dispersal and replacement. Clinical WGS, often based on tiled-amplicon protocols or metagenomic approaches, supports lineage assignment, phylogenetic reconstruction and the detection of mutations potentially relevant to diagnostics, vaccines and therapeutics. Its utility depends on specimen selection, viral RNA concentration, sequencing quality, turnaround time and transparent sharing of associated epidemiological metadata. A comparison across 189 countries demonstrated substantial disparities in sequencing coverage and submission timeliness between high-income and lower-income settings [55]. These inequities can delay recognition of variant emergence and distort inferred origins by preferentially sampling better-resourced populations.

The transition from broad diagnostic testing to more selective testing has increased the importance of sampling design. A sequence dataset concentrated among hospitalized individuals, travelers or selected health facilities cannot automatically represent the circulating virus population. Changes in test utilization, specimen eligibility and sequencing throughput can cause apparent lineage shifts even when the underlying epidemic composition changes more gradually. National surveillance in the United States documented multiple transitions between XBB- and JN.1-derived variants during 2023–2024, illustrating the operational importance of sustained monitoring and consistent lineage definitions [56]. Sequence proportions should be interpreted alongside sample counts, collection dates, geographical coverage and uncertainty rather than as exact population frequencies.

Wastewater sequencing provides an important complement to individual clinical specimens. Environmental samples integrate contributions from many infected individuals, including people who do not seek testing, and may detect circulating lineages absent from the contemporaneous clinical sequence dataset. A South African investigation demonstrated broad concordance between wastewater and clinical genomic surveillance while identifying additional lineages and providing signals of lineage turnover [57]. Nevertheless, environmental RNA is frequently fragmented and comprises a mixture of viral haplotypes; distinguishing shared mutations from complete genomes requires careful computational deconvolution. The viral abundance measured in wastewater does not directly indicate individual-level infectiousness or the clinical severity of circulating strains.

A 2026 review of wastewater genomic surveillance highlighted persistent limitations involving sample concentration, sequencing coverage, variant deconvolution, analytical validation and reproducibility [58]. These problems become particularly difficult when

genetically similar descendants coexist, when lineage-defining mutations are shared, or when declining clinical sequencing reduces the availability of independent reference data. Wastewater and clinical surveillance should therefore be treated as complementary rather than interchangeable systems. A robust genomic program should combine sequence quality assurance, representative sentinel collection, environmental measurements where feasible, and systematic reconciliation of variant calls between data sources.

Surveillance outputs require timely clinical and public health interpretation. Variants with unusual antigenic phenotypes should trigger proportionate evaluation of neutralization, diagnostic assay performance, antiviral susceptibility and relevant epidemiological endpoints; they should not automatically be assumed to cause more severe disease. Sustainable sequencing infrastructure is particularly important in low- and middle-income countries, where uneven coverage creates blind spots in global evolutionary inference. Beyond SARS-CoV-2, the enduring contribution of pandemic-era genomic capacity may be its integration into coordinated surveillance of multiple respiratory pathogens and other emerging infections [55,58].

Transmission dynamics of SARS-CoV-2

Modes and mechanisms of SARS-CoV-2 transmission

SARS-CoV-2 transmission is a multistep process in which virus-containing material must be emitted from an infected individual, remain biologically competent during transport, reach a susceptible recipient, and establish productive infection at an accessible mucosal site. Respiratory particles expelled during breathing, speech, coughing, singing, and other expiratory activities provide the principal vehicle for person-to-person transmission. These particles occur along a continuum of sizes and are variably transported through the surrounding air; consequently, strict separation into large ballistic 'droplets' and small 'aerosols' does not adequately capture their physical behavior or the opportunities for inhalational exposure. Evidence from outbreaks, aerosol physics and virological investigations supports an important role for airborne transmission in indoor environments [3,59].

The dose inhaled by an exposed individual is jointly determined by the infectious aerosol emission rate, local air movement, exposure duration, respiratory activity, particle removal, and the fraction of inhaled virus that remains infectious. Close-range exposure is generally efficient because concentrations can be high within an infected person's expiratory plume; nevertheless, shared-room transmission may also occur beyond conventional close-contact distances when infectious aerosols accumulate in poorly ventilated air. Detection of viral RNA on a surface or in air establishes contamination but does not demonstrate that replication-competent virus was present at a dose sufficient for transmission. Conversely, inability to culture virus from a sample does not prove that the air was noninfectious, because collection and processing can reduce viral viability [59].

Controlled laboratory experiments demonstrated that SARS-CoV-2 can retain infectivity in experimentally generated aerosols and on several surface materials under selected environmental conditions [60]. Such measurements establish biological plausibility rather than the frequency of transmission by each pathway in ordinary settings. Subsequently, viable SARS-CoV-2 was recovered from hospital-room air samples collected several meters from patients, demonstrating that infectious particles can be suspended and transported in room air without a conventional aerosol-generating medical procedure [61]. The combination of laboratory viability, environmental culture and epidemiological patterns offers stronger evidence for inhalational transmission than any single experimental modality.

Direct deposition of respiratory particles onto ocular, nasal or oral mucosa, and indirect transfer from contaminated hands or objects, remain biologically plausible pathways. Their relative contributions are difficult to distinguish through observational studies because individuals exposed to contaminated surfaces are often simultaneously exposed to shared indoor air. In particular, environmental RNA measurements cannot be translated into a numerical fraction of infections attributable to fomites. Accordingly, transmission models should avoid assigning precise route-specific proportions without route-discriminating evidence. A mechanistically coherent prevention strategy considers inhalation, proximity, ventilation, source

control, and hand hygiene together, while prioritizing interventions according to the strength of the available evidence [3,60,62].

Transmission should also be distinguished from clinical severity. An individual with mild disease may generate sufficient infectious aerosols to infect others, whereas a hospitalized patient with severe lower respiratory tract involvement is not necessarily more infectious late in illness. At a population level, viral characteristics interact with human contacts, immunity, built environments, and intervention practices to determine epidemic growth. Consequently, the 'transmissibility' of a lineage cannot be represented by a fixed biological property independent of its epidemiological and immunological context [7,59].

Airborne transmission and respiratory particle dynamics

The airborne pathway integrates particle generation, transport, dilution, deposition and inhalation. Small particles can remain airborne for prolonged periods, and even larger initially emitted droplets undergo evaporation that alters their size and trajectory. Their spatial distribution depends on ventilation supply and exhaust, turbulence, thermal gradients, air-cleaning systems and occupant movement. Relative humidity, temperature and ultraviolet radiation may modify particle persistence or viral inactivation, but effects observed under controlled conditions do not translate directly into universal risk thresholds. Indoor air is therefore best understood as a dynamically mixed exposure medium rather than as a fixed zone divided at a prescribed interpersonal distance [59,62].

Outbreak reconstructions were central to recognizing airborne spread. The Skagit Valley Chorale outbreak, in which numerous infections followed a single choir rehearsal, was difficult to explain by direct contact with each infected participant. A mechanistic reconstruction found that inhalation of aerosols in a shared indoor environment was consistent with the observed clustering, although the source-strength estimate depended on assumptions about the exposure circumstances [63]. The example illustrates why prolonged vocalization and limited air replacement can amplify risk, but does not imply that every singing event has the same attack rate or that a single modeled mechanism has been experimentally demonstrated in every case.

Transmission is highly heterogeneous between infected individuals and exposure events. Analysis of outbreak-size distributions identified substantial overdispersion, indicating that a relatively small proportion of infections may generate a disproportionate fraction of secondary cases under some conditions [64]. This pattern reflects a combination of variation in infectious aerosol emission, timing of contacts, susceptibility of exposed persons and environmental opportunity. Thus, the notion of 'superspreading' should be applied to the interaction between person and setting, rather than treated as an immutable attribute of selected patients.

In a systematic review and meta-analysis of community indoor environments, the pooled secondary attack rate (SAR) was 20.4% (95%CI: 12.0–32.5%), with higher estimates in singing, entertainment and fitness settings, although CIs were wide and individual investigations were heterogeneous [65]. Such setting-specific estimates are descriptive rather than transportable constants: outbreak studies often select events that were detected precisely because several infections occurred. Nevertheless, the consistency of indoor clustering across settings supports exposure reduction through adequate outdoor air exchange, appropriate filtration, reduced crowding, and shorter high-risk contact duration.

Measurements of exhaled breath aerosols have provided direct virological evidence. A study comparing variant periods detected greater aerosolized viral RNA shedding with Alpha, Delta and Omicron infections than with earlier strains and recovered culturable SARS-CoV-2 from some vaccinated and boosted participants [66]. These findings establish that breakthrough infections can produce infectious exhaled particles, but do not imply equivalent infectiousness across vaccinated and unvaccinated persons after accounting for infection probability, illness timing and immunity. Aerosol production varies markedly across individuals and activities; a single nasopharyngeal RNA measurement may consequently be a poor surrogate for the dose emitted into shared air.

Environmental and intervention studies further suggest that measures acting on particle emission and concentration can reduce exposure. In a Swiss secondary-school study conducted during Omicron circulation, a combination of environmental measurements, molecular sampling and Bayesian modeling suggested lower modeled transmission risk during mask-wearing periods,

whereas the effect attributable to portable air cleaners was too uncertain to establish a reduction under the studied conditions [67]. The finding should not be generalized as proof that filtration is ineffective, because equipment performance, placement, background ventilation, and statistical power differ greatly among classrooms. Together, mechanistic and observational findings favor a layered indoor-air approach without assuming that any single engineering control eliminates risk [62,67].

Symptomatic, presymptomatic and asymptomatic transmission

An important epidemiological characteristic of SARS-CoV-2 is the temporal dissociation between symptoms and the capacity to transmit infection. Viral replication in the upper respiratory tract can precede clinical manifestations; consequently, individuals may expose others before recognizing that they are infected. An early analysis combining serial throat-swab results and infector–infectee data estimated that infectiousness peaked around symptom onset and attributed approximately 44% (95%CI: 25–69%) of secondary infections to presymptomatic transmission in the particular settings studied [68]. This estimate was model dependent and should not be interpreted as an invariant proportion across all variant periods, vaccination histories or testing strategies.

Controlled human challenge experiments strengthened inference about early infection dynamics by observing viral replication after a known exposure time. In a study involving selected healthy young adults, detectable viral shedding developed rapidly following inoculation, and high upper respiratory tract viral loads were found in some individuals with mild or limited symptoms [69]. These observations are biologically informative but were obtained under specific challenge conditions with selected participants and an early SARS-CoV-2 strain. They should not be extrapolated directly to older persons, immunocompromised individuals, or currently circulating antigenically distinct lineages.

Prospective community cohorts that collected daily respiratory specimens and symptom reports clarified the imperfect overlap between symptom onset and viral cultivability. In the Assessment of Transmission And Contagiousness of COVID-19 Contacts (ATACCC) investigation, infectious-virus detection varied across illness stages, and symptom-based classification alone could not precisely identify the beginning or end of the infectious window for every participant [70]. The study highlights a crucial conceptual distinction: incubation period is the time from infection to symptom onset, whereas the latent period ends when onward transmission first becomes possible. Because the latent period can end earlier than the incubation period, an infected person may be infectious while still presymptomatic.

Asymptomatic infections also contribute to dissemination, although the magnitude of transmission from individuals who never develop symptoms is difficult to measure. Studies that label cases 'asymptomatic' at diagnosis may inadvertently include persons who subsequently develop symptoms, and contact-tracing datasets can preferentially detect symptomatic index cases. Furthermore, an absence of reported symptoms does not ensure an absence of respiratory shedding. Daily longitudinal sampling demonstrated substantial inter-individual variation in infectious virus shedding and a weak correspondence between a single respiratory sample and total infectiousness over the course of illness [71]. These methodological limitations warrant careful differentiation between truly asymptomatic infections and infections sampled before symptom onset.

The combined implication is that case identification based exclusively on symptom screening is insufficient to intercept all transmissions, particularly during periods of intense community circulation. Timing of exposure and testing remains important even when persons have few symptoms. At the same time, the relative frequency of presymptomatic and asymptomatic spread may differ among vaccinated or previously infected populations because immunity can alter symptom expression, viral kinetics and the likelihood that infections are detected. In contemporary practice, the evidence supports flexible, risk-adapted prevention that recognizes transmission can precede a clinical diagnosis [68–71].

Viral shedding, infectious period and transmission potential

Viral RNA shedding, antigen positivity and infectious-virus shedding describe overlapping but distinct processes. Reverse transcription polymerase chain reaction (RT-PCR) detects viral

nucleic acid that can persist after the disappearance of replication-competent virus; viral culture provides more direct evidence of viable virus but is sensitive to sampling site, transport conditions, cell system, and the timing of collection. Antigen tests detect viral proteins and may correlate more closely than RT-PCR with contemporaneous culture positivity during portions of acute illness, but a negative result cannot categorically exclude infectiousness. Consequently, no single diagnostic threshold accurately determines whether every individual can transmit at a given time [70,71].

In a longitudinal study of nonhospitalized adults, respiratory viral RNA remained detectable substantially longer than culturable virus, while nucleocapsid antigen showed a strong association with culture positivity [72]. These results illustrate why interpreting all PCR-positive results as evidence of an ongoing infectious state overestimates transmission risk, particularly in late convalescence. However, the time to culture negativity differs across individuals, and population averages should not be transformed into universal isolation cutoffs without accounting for immunocompromising conditions, illness severity, specimen quality and ongoing respiratory symptoms.

A 2026 community-cohort analysis linked the timing of peak upper respiratory tract viral load with peak symptom burden and found changes in symptom patterns around the onset of viral cultivability in a subset with culture data [73]. Symptoms therefore convey useful information about the stage of infection at a population level, even though they remain insufficiently reliable for determining infectiousness in an individual patient. The strength of these associations is affected by the symptom instrument used, follow-up frequency, the selection of culture samples and the extent to which infection was observed from its earliest phase.

The effect of antiviral treatment on shedding adds further complexity. Prospective household studies published in 2026 assessed infectious viral shedding and virological rebound among adults with and without oral antiviral use [74]. Such observational comparisons must be interpreted in the context of confounding by indication, differences in disease risk, timing of treatment initiation and frequency of testing. Viral rebound, a renewed positive test, and a second period of culture-positive shedding are not synonymous outcomes; nor does rebound by itself prove that a secondary infection was transmitted. Treatment-related effects on onward transmission require studies designed to capture secondary cases, rather than relying exclusively on within-host viral measurements.

The timing of transmission also governs epidemic growth. Serial interval is the elapsed time between symptom onset in a primary case and symptom onset in a secondary case, whereas the generation interval is the time separating their infections. A meta-analysis reported pooled mean serial intervals of 3.9 days (95%CI: 3.4–4.3) for Delta and 3.2 days (95%CI: 2.9–3.5) for Omicron, with substantial variation across source studies [75]. Such estimates are shaped by immunity, case ascertainment and interventions; the serial interval may be negative when symptoms appear in the secondary case before those in the identified infector.

Variant-specific comparisons must additionally account for epidemic growth and contraction. Reanalysis of contemporaneous Delta and Omicron datasets showed that failure to correct for changing growth rates can distort inferred incubation and generation intervals [76]. This problem is especially consequential when one lineage is replacing another, because individuals observed at the same calendar time may originate from infections occurring at different earlier times. A shorter observed interval should therefore not be interpreted solely as proof of intrinsically faster replication. Combining contact tracing, viral cultures and appropriately specified transmission models offers a more credible basis for comparing viral lineages [75,76].

Environmental, behavioral and occupational determinants of transmission

Environmental transmission risk is governed by an interaction between pathogen emission, accumulated airborne concentration and exposure opportunity. Indoor crowding, limited outdoor air replacement, long occupancy duration, vocal activities and high exertion commonly coincide in settings associated with SARS-CoV-2 clusters. Architectural characteristics, climate, air recirculation, filtration efficiency, ventilation maintenance and occupancy schedules further modify exposure. A systematic review of indoor air quality and respiratory virus transmission identified substantial heterogeneity in environmental measurements and noted important gaps

in simultaneous assessment of air quality, virological outcomes and energy use [77]. Engineering evidence should consequently be interpreted in relation to the actual setting rather than reduced to a universal ventilation threshold.

Ventilation and respiratory protection address different points in the transmission pathway. Outdoor air exchange and appropriately designed filtration reduce the concentration of potentially infectious airborne particles; well-fitting respiratory protection limits inhalation exposure; and source control reduces particle release by infected individuals. These interventions may provide complementary rather than perfectly substitutable benefits. Carbon dioxide measurements can identify inadequate outdoor-air exchange under certain occupancy conditions, but CO2 is not a direct measurement of infectious aerosol concentration and may be misleading when occupancy patterns, filtration or air mixing differ. Improvements in indoor air should also account for feasibility in schools, public transport, hospitals, workplaces and resource-limited buildings [62,67,77].

Behavioral factors influence who is exposed, where and for how long. Repeated household contact, social gatherings, communal meals, shared accommodation and occupational proximity can create overlapping exposure networks. Quantifying each factor independently is challenging because masking, ventilation, duration and the probability that an infected person is present are not randomly distributed. Moreover, the relative importance of a given behavior may change when vaccination, previous infection or a new variant modifies population susceptibility. Therefore, comparisons of transmission risk across occupations or settings should adjust for confounding and consider the prevailing epidemiological period rather than attributing risk solely to one activity [65,77].

The contribution of contaminated surfaces should be interpreted cautiously. A prospective household investigation associated SARS-CoV-2 RNA on hands and frequently touched surfaces with infection risk among contacts, and viral sequencing was consistent with some within-household transmission links [78]. Nevertheless, surface contamination can be a marker of the amount of virus shed and of shared close-contact exposures, rather than proof that infection occurred after hand-to-mucosa transfer. Such data support hygiene as a reasonable complementary measure while leaving uncertainty about the population attributable fraction of fomite transmission. Surface RNA detection, viability experiments and directly observed infection routes are different evidentiary endpoints.

A setting-specific systematic review found marked differences in observed secondary attack rates, with household exposure generally exceeding lower-risk casual contacts and with duration of contact playing an important role [79]. These estimates are prone to differential testing and exposure misclassification. In particular, occupational risk can be overestimated when community acquisition is not measured, or underestimated when frequent asymptomatic screening identifies infections whose sources are uncertain. Prevention programs should combine setting-specific risk assessment, adequate ventilation, appropriate personal protective measures and surveillance procedures proportionate to the risk of severe outcomes in exposed populations [62,79].

Household, community, healthcare-associated and institutional transmission

Households remain a central setting for SARS-CoV-2 transmission because proximity, prolonged contact and shared indoor air are difficult to avoid. An early systematic review involving 54 studies and 77,758 participants estimated a household secondary attack rate of 16.6% (95%CI: 14.0–19.3%), while documenting pronounced heterogeneity by index-case symptoms, household relationships and contact age [80]. These estimates described predominantly early-pandemic conditions with comparatively little pre-existing immunity and should not be generalized uncritically to later Omicron periods.

An updated meta-analysis found an estimated household SAR of 42.7% (95%CI: 35.4–50.4%) in the Omicron studies available at the time, higher than estimates reported for several earlier variants [81]. Nevertheless, comparisons between variant-specific SARs confound viral characteristics with changing immunity, test sensitivity, study design, exposure patterns and the likelihood that more than one household member acquired infection outside the home. Such rates represent the proportion infected during follow-up rather than a direct measure of intrinsic viral

fitness. In addition, late ascertainment of an index case can cause co-primary infections to be misclassified as transmissions from that individual.

Combining frequent testing with viral sequencing can refine source attribution. In a Massachusetts household cohort during Omicron circulation, the estimated SAR differed substantially according to whether the analysis considered all follow-up infections, households with a single baseline-positive case or only genomically and epidemiologically probable transmission events [82]. This finding underscores the importance of separating secondary transmission from external introductions before comparing populations or inferring the effect of preventive interventions. Genomic identity alone may be insufficient to resolve the source when the same highly prevalent lineage is circulating in the community.

Recent evidence from Southeast Asia indicates that household transmission remained relevant even after widespread vaccination. A prospective cohort in Chiang Mai, Thailand, encompassing exposures between 2022 and 2024, reported an SAR of 33% (95%CI: 24–44%), while a phylogenetically supported estimate was 25% (95%CI: 17–35%) [83]. Lower index-case viral load and evidence of prior immunity in contacts were associated with lower transmission risk. Although these findings are informative for middle-income settings, their generalizability depends on household selection, community incidence, previous immunity and the particular Omicron descendants circulating during follow-up.

Institutional transmission presents distinct challenges. Healthcare facilities may combine susceptible patients, prolonged indoor exposure, immunocompromising conditions and repeated close-contact care. Long-term-care facilities, shelters, prisons, schools and other congregate settings can also support transmission chains when introductions coincide with susceptible contact networks. Viral genome sequencing can identify plausible hospital clusters or exclude proposed transmission links, but it must be integrated with specimen collection dates, patient movement, staff assignments and contextual epidemiology. A systematic review of 97 studies concluded that sequencing frequently informed outbreak reconstruction, yet evidence that genomic sequencing alone reduced hospital-acquired COVID-19 incidence was lacking [84].

These findings point toward a common investigative principle: setting-specific transmission estimates are only as robust as their source attribution and ascertainment. Within-household or within-institution exposure can be inferred incorrectly when index-case definition relies solely on the first positive test, when sequencing coverage is sparse, or when external introductions are frequent. Well-designed studies should document symptom timing, systematic contact testing, vaccination and infection history, occupancy patterns, genomic relatedness where feasible and a pre-specified definition of probable secondary infection. Accurate characterization is essential for comparing intervention effectiveness across locations and periods [82–84].

Effects of viral evolution and population immunity on transmission dynamics

The transmission advantage of an emerging SARS-CoV-2 lineage is jointly shaped by viral phenotype and the immune landscape in which it circulates. Changes in spike-mediated receptor engagement, cell entry, antigenicity and exhaled virus shedding can influence the probability that exposure results in infection. At the same time, a lineage with stronger antibody escape may grow rapidly in a population with substantial prior immunity without necessarily exhibiting greater intrinsic infectivity in an immunologically naive host. The successive replacement of Omicron descendants therefore requires integrated interpretation of molecular virology, serology, population contact behavior and lineage-specific epidemiological data [7,47,48,66].

Vaccination and previous infection affect transmission through at least two epidemiologically distinct mechanisms: susceptibility to infection and infectiousness conditional on breakthrough infection. These should not be conflated with vaccine effectiveness against severe disease, which may remain comparatively robust despite diminished protection from infection. Early systematic evidence indicated that vaccination was associated with lower susceptibility and onward transmission, but that the estimated effects against Omicron infection were less pronounced than those reported against earlier variants [81]. The magnitude and duration of protection also depend on vaccine products, recency of immunization, prior infection history, age and antigenic distance between the immunizing and circulating variants.

In two prospective multisite United States household cohorts covering 2021–2023, the risk of infection among exposed contacts was lower in those with hybrid immunity than among

persons without previous vaccination or infection; a more recent immunizing event was also associated with greater protection [85]. These associations support a dynamic rather than binary model of immune protection. However, they cannot be assumed constant for variants circulating several years later, and the comparison of immunity strata may remain affected by differences in exposure, testing behavior, and previous infection ascertainment.

Population-level transmission may persist despite extensive prior infection and vaccination because sterilizing protection against genetically evolving respiratory viruses is incomplete and may wane. The effective reproduction number reflects this residual susceptibility together with contact rates, transmission probability and the duration of infectiousness. Apparent seasonal waves can additionally reflect changes in indoor congregation, respiratory-virus ecology, school calendars and population immunity; their relative contributions vary by region. Consequently, assigning observed peaks solely to a particular new variant or to climate without appropriate comparative analysis risks ecological overinterpretation [23,27,75].

The central implication for 2026 is that SARS-CoV-2 transmission cannot be represented by a single stable parameter or confined to symptomatic individuals and short-range contacts. Sustainable surveillance and prevention should incorporate changes in variant prevalence, infection-derived and vaccine-derived immunity, infectiousness around symptom onset, aerosol exposure in indoor environments and continued risk in vulnerable settings. Because prospective transmission studies have become less frequent as community testing declined, contemporary estimates should include explicit uncertainty and transparent descriptions of sampling and denominators. These epidemiological considerations provide the foundation for subsequent discussion of COVID-19 pathogenesis and clinical consequences [30,31,83,85].

Pathogenesis and host immune responses

Viral entry, cellular tropism and tissue distribution

The pathogenesis of COVID-19 begins with productive SARS-CoV-2 infection at susceptible mucosal sites and is subsequently determined by the balance between viral replication, antiviral defense and host-mediated tissue injury. Viral entry is mediated principally by engagement of the spike protein with ACE2, followed by proteolytic activation of spike and fusion at the plasma membrane or within an endosomal compartment. These entry mechanisms, including receptor binding and subsequent membrane fusion, are illustrated in **Figure 3**. TMPRSS2 supports cell-surface entry in susceptible epithelial cells, whereas alternative endosomal pathways can contribute in particular cellular contexts. Entry-route dependence differs among viral lineages and experimental systems; therefore, receptor expression alone cannot be interpreted as evidence of productive infection in every organ expressing ACE2 [37,45,46].

The upper respiratory tract constitutes an important site of early replication. Human airway epithelial studies identified a pronounced proximal-to-distal gradient of ACE2 expression and susceptibility, with infected ciliated epithelial cells in conducting airways and type II pneumocytes in distal lung specimens. This spatial distribution provides a plausible biological basis for efficient respiratory shedding and, in patients with progressive pulmonary disease, extension of infection toward the gas-exchange surface [86]. However, the magnitude and spatial distribution of viral replication are modified by inoculum, variant-specific entry biology, mucosal immunity, and the underlying integrity of the respiratory epithelium. Findings obtained with ancestral SARS-CoV-2 cannot automatically be extrapolated to contemporary Omicron descendants.

Although the respiratory system represents the principal site of clinically consequential infection, extrapulmonary viral susceptibility has been demonstrated. Human intestinal organoids and epithelial models support productive infection of enterocytes, providing mechanistic plausibility for gastrointestinal symptoms and fecal viral RNA detection [87]. Autopsy investigations involving extensive tissue sampling detected SARS-CoV-2 RNA and, in some early samples, evidence of replication across several extrapulmonary sites, including neural tissue. These findings established that systemic viral dissemination may occur, particularly in severe or fatal illness, but did not demonstrate that every RNA-positive compartment sustains ongoing replication or clinically important direct cytopathic injury [88].

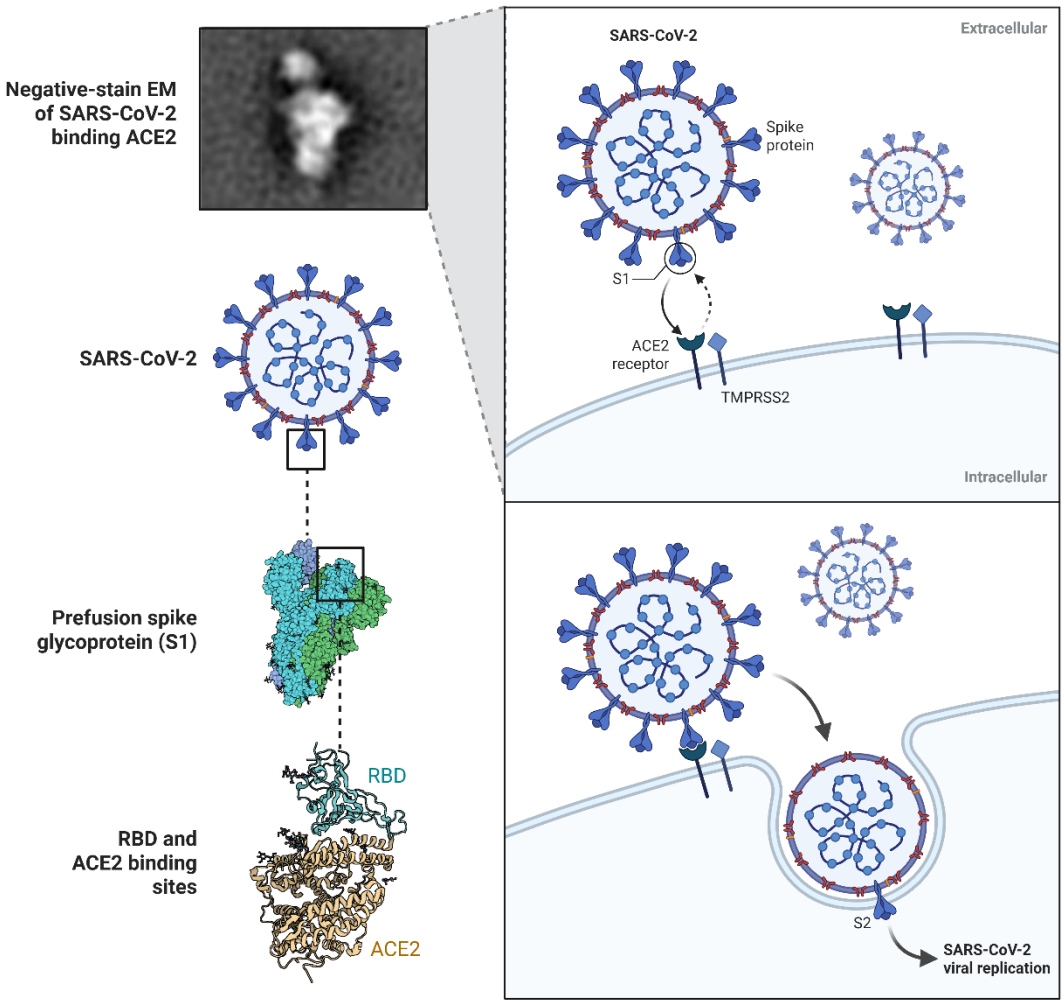


Figure 3. SARS-CoV-2 targeting of ACE2 and host-cell entry. Viral entry begins with binding of the spike (S) protein receptor-binding domain (RBD) to angiotensin-converting enzyme 2 (ACE2) on the host-cell surface. Proteolytic activation of spike, including by transmembrane serine protease 2 (TMPRSS2), facilitates fusion at the plasma membrane or entry through the endosomal pathway. The left panels illustrate the molecular interaction between the spike RBD and ACE2, whereas the right panels depict receptor binding and host-cell entry leading to infection.

Interpretation of organ tropism requires a hierarchy of evidence. Positive nucleic acid amplification can reflect intact virus, residual genetic material, or contamination by infected blood and cells; immunostaining depends on reagent specificity; and viable-virus recovery, replication intermediates, or convincing spatially resolved cellular localization provide stronger evidence of productive infection. Likewise, a neurological, cardiac, or renal manifestation can arise through inflammatory, vascular, metabolic, or treatment-related mechanisms in the absence of substantial direct viral replication within that tissue. This distinction is essential when attributing multisystem disease and persistent symptoms to viral reservoirs. The strongest inference emerges from concordant virological, histopathological and clinical observations rather than the detection of viral RNA alone [88].

Innate immune responses to SARS-CoV-2 infection

Innate immune recognition provides the first coordinated barrier against SARS-CoV-2. Viral RNA is sensed by cellular pattern-recognition receptors, including endosomal Toll-like receptors and cytosolic RNA sensors, which activate transcriptional programs promoting type I and type III interferons and inflammatory mediators. Interferon signaling induces antiviral effectors that restrict viral entry, replication, and assembly, while chemokines recruit leukocytes to sites of infection. A timely localized response can limit viral amplification and enable the development of adaptive immunity without extensive tissue damage. In contrast, inadequate early antiviral

signaling may permit viral expansion, followed by disproportionate or prolonged inflammation during later disease stages [89].

SARS-CoV-2 encodes multiple proteins that antagonize different elements of interferon induction, signaling, and host gene expression. The combined effects are context-dependent and may vary with cell type, infection stage, and viral genotype. Viral antagonism can therefore coexist with strong interferon-stimulated gene expression in surrounding uninfected cells, creating apparently discordant tissue and blood signatures. The temporal and spatial separation between infected epithelium and bystander immune cells is an important explanation for why severe COVID-19 cannot be characterized simply as either an interferon-deficient or an interferon-excess state [90].

Patient-based investigations have identified impaired circulating type I interferon activity accompanied by enhanced proinflammatory signaling in some individuals with severe disease. In an integrated analysis, reduced interferon-α and interferon-β activity was associated with persistent blood viral RNA and elevated inflammatory mediators, including interleukin-6 and tumor necrosis factor pathways [91]. Mechanistic evidence for the importance of interferon-mediated defense was strengthened by studies of rare inborn errors affecting the Toll-like receptor 3/interferon regulatory factor 7 circuit [92] and neutralizing autoantibodies against type I interferons among patients with life-threatening pneumonia [93]. These findings identify etiologically informative subgroups rather than implying that interferon deficiency explains all severe cases.

Inflammasome activation constitutes another intersection between antiviral sensing and inflammatory injury. Studies of patients and experimentally infected cells have reported activation of inflammasome-associated pathways, with downstream processing of interleukin-1β and interleukin-18 and associations with disease severity [94]. Monocytes, macrophages, neutrophils, natural killer cells and epithelial cells contribute distinct, interacting functions to innate control and pathology. Their relative importance depends on the site of sampling, comorbidities and timing of observation. Consequently, an isolated blood cytokine measurement should not be regarded as a complete representation of pulmonary immune activity or a mechanistic diagnosis.

A coherent interpretation of innate immunity must distinguish protective antiviral signaling from later immunopathology. Responses that are insufficient or delayed during early viral replication may be maladaptive if stimulated after substantial tissue injury has occurred. Conversely, suppression of inflammation during uncontrolled early infection could impair viral clearance. These temporal dependencies help explain why the biological effect of an immune-modifying intervention can depend on disease stage and why experimental antiviral or immunomodulatory results require evaluation in appropriately stratified clinical populations [90,91,95].

Adaptive immunity: humoral and cellular responses

Adaptive immunity to SARS-CoV-2 is mediated by coordinated B-cell, CD4-positive T-cell, and CD8-positive T-cell responses. Following antigen recognition and presentation, CD4-positive helper cells support germinal-center reactions and antibody maturation, cytotoxic CD8-positive cells recognize and eliminate infected cells, and B cells differentiate into antibody-secreting plasmablasts, memory B cells and longer-lived plasma cells. These functions are complementary rather than interchangeable: antibodies can neutralize extracellular virions and limit infection at susceptible surfaces, whereas virus-specific T cells contribute to control of established intracellular infection and the limitation of disease progression [96].

The magnitude of circulating antibody or lymphocyte activation is not a linear marker of protection. In acute disease, marked plasmablast expansion may coexist with severe illness, reflecting antigen burden and immune perturbation rather than superior host control. High-dimensional immune profiling identified heterogeneous immunological patterns among hospitalized patients, including markedly activated T- and B-cell compartments in some individuals and relatively muted lymphocyte activation in others. These immunotypes underline the inadequacy of treating severe COVID-19 as a single uniform inflammatory phenotype [97].

Virus-specific cellular responses are particularly relevant to early infection control. Longitudinal observations of symptomatic patients linked earlier functional SARS-CoV-2-

specific T-cell responses to faster viral clearance and milder clinical courses, although observational designs and small samples limit causal inference [98]. Epitopes targeted by T cells occur across several viral proteins, and many are less antigenically variable than prominent neutralizing antibody epitopes on spike. Consequently, loss of neutralization against a new variant does not necessarily imply comparable loss of T-cell recognition. Heterogeneity in human leukocyte antigen (HLA) restriction, immunosenescence and immunosuppressive conditions nevertheless affect the breadth and magnitude of cellular protection.

Immunological memory is established through interconnected but kinetically distinct compartments. Longitudinal studies after infection demonstrated that circulating antibody, memory B-cell, and virus-specific T-cell responses do not decline at identical rates [99]. Bone marrow sampling provided direct evidence of long-lived spike-specific plasma cells after recovery from mild infection, establishing a biological mechanism for durable antibody production despite an initial reduction from peak circulating titers [100]. Memory should not be equated with permanent sterilizing protection: previously primed immune cells may respond more rapidly after exposure even when baseline mucosal neutralizing antibody concentrations have fallen below those needed to prevent infection.

Vaccination activates overlapping mechanisms without requiring the potentially unpredictable morbidity of infection. Studies following messenger RNA vaccination demonstrated persistent memory B and T cells for at least several months, including memory B cells with cross-reactivity to early variants, despite decreasing circulating antibody concentrations [101]. Interpretation in 2026 must account for the accumulation of vaccination, infection, and reinfection histories that have diversified individual immune repertoires. Importantly, memory measured in peripheral blood does not fully represent resident immune populations in nasal, airway and lung tissues, and the relationship between systemic measurements and mucosal protection remains incompletely resolved.

Dysregulated inflammation and cytokine-mediated tissue injury

The clinical transition from a predominantly virus-driven illness to severe inflammatory lung disease involves a failure to terminate injurious host responses while preserving effective antiviral defense. Infected epithelial cells, resident macrophages, recruited monocytes, and other leukocyte populations release cytokines and chemokines that can amplify vascular permeability, epithelial injury, and leukocyte recruitment. Interleukin-6, interleukin-1 family mediators, tumor necrosis factor, and interferon-associated pathways participate in overlapping inflammatory networks. The term cytokine storm is frequently used, but it risks suggesting a homogeneous syndrome or that circulating cytokine concentrations necessarily exceed those observed in other critical illnesses [102].

Monocyte and macrophage activation has been implicated in sustaining tissue inflammation when viral replication, epithelial damage, and inflammatory recruitment become mutually reinforcing. Macrophages may perform protective functions through phagocytosis and tissue repair while simultaneously producing mediators that aggravate lung injury under dysregulated conditions. Disease severity therefore cannot be inferred simply from macrophage abundance or a single activation marker; phenotype, location, timing, and interactions with endothelial and epithelial compartments are more informative [103].

Longitudinal analyses demonstrated that unfavorable outcomes were associated with persistent, complex immune activation rather than with one isolated cytokine abnormality. Individuals who recovered from moderate disease more often showed declining inflammatory activity and signatures compatible with tissue repair, whereas severe illness was associated with sustained activation spanning multiple immune-response programs [104]. Early immune activation may represent an appropriate response to infection, but persistence in the setting of established alveolar damage can become pathogenic. This distinction is central to understanding why therapies that suppress inflammation can be beneficial in selected patients requiring oxygen while having different risk-benefit profiles in uncomplicated early infection [11].

The immunopathological model has matured since 2020. Severe COVID-19 is not adequately explained by uncontrolled viral replication alone or by a universally defined hyperinflammatory state; rather, defective viral control, maladaptive cellular responses, vascular abnormalities and host susceptibility interact in different proportions across patients and disease stages. A review

integrating the first five years of mechanistic evidence similarly emphasized the importance of distinguishing early antiviral protection from subsequent inflammation-mediated injury and the relative resilience of cellular immunity to antigenic evolution [105]. Biomarker-based treatment personalization remains a promising objective, but inflammatory measurements require prospective validation before they can reliably direct specific immunomodulatory regimens.

Endothelial dysfunction, coagulopathy and thromboinflammation

Vascular pathology is a major contributor to severe COVID-19 and provides a mechanistic link between pulmonary inflammation, impaired oxygen exchange and systemic organ dysfunction. Lung autopsy comparisons revealed endothelial injury, microvascular thrombosis and altered vascular remodeling in fatal COVID-19, together with diffuse alveolar damage. Such observations established that severe respiratory failure is not solely a disorder of alveolar epithelial infection but also involves the pulmonary microcirculation [106]. Nevertheless, the existence of endothelial injury does not establish that productive viral infection of endothelial cells is the predominant mechanism; inflammatory activation, hypoxia, immune complexes, and coagulation abnormalities may contribute without direct endothelial tropism.

Circulating markers of endothelial activation and von Willebrand factor-associated pathways were elevated in hospitalized patients, supporting the presence of systemic endotheliopathy in some severe cases [107]. Endothelial barrier dysfunction permits inflammatory-cell trafficking and tissue edema, while exposure of procoagulant surfaces promotes thrombin generation. These abnormalities may coexist with conventional venous thromboembolism, small-vessel immunothrombosis and organ-specific microcirculatory impairment. They should not be conflated, because their anatomical distributions, diagnostic approaches and response to anticoagulation may differ.

Immunothrombosis arises from reciprocal activation of innate immunity and coagulation. Neutrophil extracellular traps, activated platelets, tissue factor pathways, complement activation, and fibrin deposition can contribute to intravascular inflammatory thrombi. Histological and functional studies linked neutrophil-platelet activation patterns and microvascular thrombi with more severe respiratory disease and coagulopathy [108]. Although such associations support a mechanistic role, they do not demonstrate that a particular biomarker or pathway can be targeted safely and effectively in all patients. The balance between thrombosis prevention and bleeding risk remains a clinical consideration distinct from establishing the mechanism of vascular injury.

Complement activation provides an additional connection between pathogen recognition, vascular inflammation and coagulation. A scoping review incorporating histopathological, observational and interventional evidence found substantial support for activation of classical, lectin and alternative complement pathways during COVID-19, with associations between activation markers and adverse outcomes [109]. However, pathway activation is not equivalent to proven benefit from complement inhibition; studies vary in sampling times, assays, and patient selection. The combined evidence supports an integrated vascular-immunological framework in which inflammation promotes thrombosis and thrombosis aggravates tissue dysfunction, particularly within the injured pulmonary microvasculature.

Mechanisms of acute respiratory distress syndrome and multiorgan injury

Acute respiratory distress syndrome (ARDS) represents the convergence of viral epithelial injury, dysregulated host inflammation, endothelial activation, and failure of the alveolar-capillary barrier. Damage to type I and type II pneumocytes compromises gas exchange and surfactant homeostasis, while leakage of protein-rich fluid into the alveolar space reduces lung compliance and promotes ventilation-perfusion mismatch. Inflammatory-cell recruitment and diffuse alveolar damage can coexist with pulmonary microthrombi, producing severe hypoxemia that is disproportionate to some conventional clinical or radiological measurements. The relative contributions of alveolar and vascular lesions differ among patients and across the temporal course of disease [86,102,106].

Progression is not necessarily a single uninterrupted cascade. Some patients develop respiratory deterioration after the peak of upper-airway viral replication, consistent with persistent host-mediated injury; others, particularly those with impaired immunity, may continue to replicate virus for prolonged periods. Secondary infections, preexisting pulmonary

disease, fluid balance, mechanical ventilation and critical-care complications further modify clinical trajectories. Thus, a pathophysiological description of ARDS must avoid attributing all respiratory failure to the direct effects of virus or to a single cytokine-driven process. Clinical outcomes reflect injury and repair pathways operating in a changing host and treatment environment [95,104,105].

Multiorgan complications can develop through combinations of hypoxemia, systemic inflammation, altered perfusion, microvascular injury, and immune-mediated mechanisms. Renal dysfunction may reflect hemodynamic abnormalities, tubular injury, nephrotoxic exposure and vascular events; myocardial injury can arise from supply-demand imbalance, thrombosis, inflammation or other cardiovascular stressors. Neurological involvement may involve vascular injury, systemic inflammatory signaling, dysautonomia and, in selected cases, direct viral presence. Autopsy identification of viral genetic material in extrapulmonary organs establishes possible dissemination but does not by itself identify the immediate mechanism of organ dysfunction [88,108].

The same distinction between tissue distribution and causation applies to prolonged symptoms after the acute phase. Persistent antigen or RNA, immune perturbation, microvascular changes and alterations in autonomic or metabolic regulation have all been investigated, yet no single mechanism explains the heterogeneity of post-COVID-19 conditions. Given differences in specimen collection and case definitions, evidence from fatal acute infection cannot automatically be generalized to ambulatory long COVID cohorts. The mechanisms and therapeutic implications of post-acute illness are considered separately in Section 9 [13,88].

Immunopathogenesis of severe and critical COVID-19

Severe COVID-19 develops when multiple protective barriers fail or when the host response becomes disproportionately injurious. Advanced age, chronic diseases, immunosuppression and host-specific biological factors alter the relative importance of viral clearance and inflammatory damage. Genetic findings have provided causal clues beyond associations with conventional clinical risk factors. WGS in a large critical-care cohort identified susceptibility loci related to interferon signaling, leukocyte biology, and other pathways, supporting at least two broad mechanisms of life-threatening disease: inadequate restriction of viral replication and enhanced propensity toward pulmonary inflammatory and vascular injury [110].

Earlier genome-wide investigations likewise implicated pathways involving interferon signaling, antiviral restriction and inflammatory regulation, including loci near IFNAR2, OAS genes, TYK2 and DPP9 [111]. Such genetic effects are probabilistic rather than deterministic: most critically ill patients do not have a single identifiable high-penetrance mutation, and common susceptibility alleles are insufficient for patient-level prediction. The epidemiological impact of host genetic findings also depends on ancestry representation, linkage disequilibrium, and replication across populations. Caution is therefore needed when extrapolating genetic effect estimates from one ancestry group to others.

Rare monogenic interferon defects and neutralizing anti-interferon autoantibodies provide more direct examples of impaired antiviral immunity associated with critical pneumonia [92,93]. These mechanisms may be particularly important in selected patients with otherwise unexplained severe disease. In contrast, hyperactive inflammatory circuits, endothelial injury and immunothrombosis may dominate later phases in other patients [104,108]. Differences in the time from symptom onset, variant exposure, vaccination history, and baseline immunity further complicate attempts to assign a uniform molecular signature to severe illness.

The response to systemic corticosteroids among appropriately selected hospitalized patients requiring respiratory support provides clinical evidence that inflammation contributes materially to adverse outcomes [11]. However, therapeutic responsiveness does not prove that every patient shares an identical cytokine profile or that immune suppression is beneficial at all stages. A more useful conceptual framework separates early antiviral competence, subsequent immune regulation, and pulmonary vascular integrity while recognizing their interactions. Prospective immune phenotyping integrated with virological data and host characteristics may ultimately support better subgroup-specific treatments, but such approaches have yet to replace evidence-based clinical severity assessment [105,110].

Immunity after infection, vaccination and hybrid exposure

By 2026, immunological responses to SARS-CoV-2 generally reflect multiple antigenic encounters rather than an isolated primary infection. Vaccine doses, infections caused by different lineages, and the intervals between exposures shape the magnitude, specificity, and maturation of adaptive responses. Hybrid immunity, conventionally defined as immunity acquired through both infection and vaccination, can broaden antibody and cellular repertoires, although the resulting protection varies according to exposure order, antigenic distance, time elapsed, and host characteristics. This breadth should not be interpreted as complete protection against reinfection or as a justification for deliberate infection [96,101].

A systematic review and meta-regression of pre-2023 studies found that hybrid immunity was associated with comparatively durable protection against hospitalization or severe disease from Omicron, whereas protection from reinfection waned more substantially over time [112]. Such estimates represent specific populations, variants, and periods, and cannot be applied unmodified to later descendants with additional immune-escape mutations. The biological distinction is nevertheless consistent with the different thresholds and kinetics required to prevent acquisition versus to prevent clinically severe disease after infection has occurred.

Immune imprinting describes the capacity of earlier antigenic encounters to shape subsequent responses, sometimes favoring recall against conserved epitopes at the expense of new variant-specific specificities. Experimental and human serological investigations have shown that repeated exposure to Omicron antigens can partially overcome ancestral-strain imprinting and generate broader responses, although the degree of change depends on the exposure history and assay system [113]. Imprinting is therefore neither an absolute obstacle nor an invariably beneficial process; its interpretation requires antigenic, cellular and epidemiological context.

Hybrid and repeatedly boosted immunity also underscores the importance of distinguishing serum responses from mucosal immunity. Systemic immunoglobulin G and circulating T cells may reduce progression to severe disease even when infection begins in the upper airway, whereas local neutralizing antibodies and tissue-resident cellular responses are more directly positioned to intercept infection at entry sites. These compartments are differently sampled and may have distinct durability. Longitudinal studies combining nasal and systemic immunity with virologically confirmed breakthrough infections are needed to quantify how these mechanisms interact in populations whose exposure histories are increasingly heterogeneous [99,101,113].

Immune escape, waning immunity and correlates of protection

Protection against SARS-CoV-2 is multidimensional. Neutralizing antibodies are closely related to protection against symptomatic infection, whereas B-cell recall, virus-specific T cells and other immune mechanisms contribute to reducing viral burden and disease severity. Across vaccine studies, neutralizing antibody concentrations were strongly associated with protection from symptomatic disease, although the relationship was estimated under defined assay, variant, and population conditions [114]. These results support neutralization as an important correlate of protection but not as an invariant individual-level threshold that guarantees immunity.

A prespecified immune-correlates analysis of the mRNA-1273 efficacy trial found associations between vaccine-induced binding and neutralizing antibody responses and subsequent COVID-19 risk [115]. Such correlates can assist the interpretation of vaccine immunogenicity and potentially reduce reliance on very large clinical trials for every updated antigen. However, assay standardization, protection endpoint, age, immune status, prior infection history, and antigenic match remain critical. Correlations observed for symptomatic ancestral-strain infection should not automatically be treated as validated surrogates for protection against severe disease from later variants.

Waning immunity is similarly endpoint specific. Circulating neutralizing antibody concentrations commonly decline after an immunizing event, while memory B cells, long-lived plasma cells and T-cell memory can persist with different kinetics [99–101]. Thus, lower antibody titers months after vaccination do not mean that protective immunity has disappeared. Conversely, preserved memory does not prevent all infections because the time required to mobilize a recall response may exceed the short interval during which a rapidly replicating respiratory virus establishes infection. Protection against acquisition is therefore generally more vulnerable to waning and antigenic drift than protection against severe outcomes [112,114].

Viral evolution further complicates immune-correlate interpretation. Mutations altering spike antigenicity can reduce the neutralizing activity of antibodies elicited by earlier exposures, while many T-cell epitopes remain relatively conserved. Contemporary variant-specific measurements should be interpreted with attention to assay platform, antigenic comparison, epidemiological setting, and the distinction between intrinsic viral properties and selection in immune populations [7,48,51]. Laboratory neutralization escape is informative but cannot by itself establish a change in hospitalization risk or population transmission advantage.

A major research priority is the development of harmonized, longitudinal correlates encompassing mucosal antibodies, functional B-cell memory, polyfunctional T-cell responses and host vulnerability. These measures should be assessed against rigorously defined endpoints, including laboratory-confirmed infection, symptomatic illness, hospitalization and death. Integration of immunological data with genomic surveillance and appropriately controlled population studies offers a more robust basis for next-generation vaccine evaluation than reliance on a single antibody measurement. Collectively, current evidence positions immune protection as dynamic, compartmentalized, and strongly conditioned by time, viral evolution, and clinical risk [96,105,114,115].

Clinical manifestations and disease severity

Incubation period and clinical course

COVID-19 encompasses a continuum of illness ranging from clinically silent infection to life-threatening respiratory failure and systemic complications. Clinical expression is determined by interactions among viral properties, pre-existing immunity, age, comorbidities, and the host response. Accordingly, symptom frequencies derived from the first pandemic waves should be treated as period-specific estimates rather than fixed biological characteristics of SARS-CoV-2. Early multicentre investigations established fever, cough, fatigue and dyspnoea as common manifestations among patients receiving medical care, whereas atypical or initially non-respiratory presentations were also documented [2,116,117].

After acquisition of infection, an incubation period precedes the appearance of symptoms in symptomatic individuals. Its distribution has shifted across variant periods, and clinical presentation is additionally influenced by vaccination and previous exposure. The absence of symptoms at one assessment cannot establish that an infected person will remain asymptomatic, particularly when follow-up is short. In the early pandemic, progression from initial systemic or upper respiratory symptoms to dyspnoea and hypoxemia was frequently observed during the subsequent week among those who developed pneumonia; this temporal trajectory reflected cohorts selected for hospitalization and should not be generalized to all infections or contemporary immune populations [2,118].

Large hospital-based studies illustrated the heterogeneity of initial presentation. A prospective investigation of 20,133 patients admitted across the United Kingdom demonstrated substantial variation in age, comorbidity burden, and presenting complaints, emphasizing the importance of integrating clinical assessment with risk stratification. A United States series of 5,700 hospitalized individuals similarly documented frequent cardiometabolic comorbidities and variable fever or oxygen requirements at triage [117,119]. These studies remain essential for characterizing the untreated and incompletely immune population during the ancestral-virus period, but their crude fatality proportions cannot be interpreted as present-day risks.

Several clinical courses may be distinguished conceptually: infection without symptoms, self-limited upper respiratory illness, lower respiratory tract disease requiring hospital-level monitoring, progressive hypoxemic respiratory failure, and critical illness accompanied by multiorgan dysfunction. These categories are dynamic. An initially stable patient may deteriorate, whereas other patients recover without radiographic pneumonia or supplemental oxygen. Clinical severity assessment therefore requires serial evaluation of respiratory status, physiological reserve, and complications rather than reliance on a single symptom or positive virological test [116–119]. The principal clinical manifestations and complications across organ systems, including selected special-population and postinfectious presentations, are summarized in **Table 3**.

Table 3. Clinical manifestations and complications of COVID-19 across organ systems

System or clinical phenotype	Common acute manifestations	Important complications and prognostic considerations	References
General/systemic	Fever, fatigue, myalgia, headache, anorexia; some cases remain asymptomatic.	Symptom patterns vary by variant and immune history; constitutional symptoms alone are nonspecific.	[2, 116]
Upper respiratory/ENT	Sore throat, nasal congestion, rhinorrhea, cough, and smell or taste disturbances.	Olfactory disturbance was prominent in early variants but is not an obligatory feature.	[116, 120]
Lower respiratory	Dyspnea, tachypnea, hypoxemia, radiographic infiltrates, and viral pneumonia.	Respiratory failure, ARDS, secondary infection, and prolonged oxygen or ventilation requirements.	[117, 121, 122]
Cardiovascular	Chest discomfort, palpitations, tachycardia, or signs of myocardial injury.	Myocarditis, myocardial injury, arrhythmias, heart failure decompensation, and thromboembolic events; distinguish myocardial injury from myocarditis.	[123, 124]
Neurological	Headache, dizziness, confusion, altered consciousness, and anosmia.	Encephalopathy, ischemic stroke, seizures, and less frequent inflammatory neurological syndromes; causality may be multifactorial.	[124, 125]
Gastrointestinal/hepatic	Diarrhea, nausea, vomiting, abdominal pain, and transaminase elevations.	Dehydration, hepatocellular injury, and complications associated with systemic illness or medications.	[87, 126]
Renal	Proteinuria, altered fluid balance, elevated creatinine, or reduced urine output in severe cases.	Acute kidney injury and associated mortality risk; hypoperfusion, inflammation, and nephrotoxicity may coexist.	[127]
Hematological/vascular	Lymphopenia, inflammatory-marker abnormalities, and coagulation disturbances.	Venous thromboembolism, pulmonary embolism, and immunothrombotic microvascular injury in severe disease.	[106, 108, 129]
Endocrine/metabolic	Hyperglycemia and metabolic decompensation, especially in patients with diabetes or critical illness.	Diabetic ketoacidosis or severe dysglycemia; corticosteroid effects and stress hyperglycemia require consideration.	[128]
Pregnancy/perinatal	Typical respiratory/systemic symptoms may occur with or without obstetric complications.	Higher risk of severe maternal illness in selected groups; evaluate preterm birth and adverse perinatal outcomes separately.	[135]
Pediatric COVID-19	Fever, cough, gastrointestinal complaints, or asymptomatic infection.	Most infections are mild; severe disease occurs in selected high-risk children.	[130, 131, 133]
Postinfectious inflammatory syndromes	Fever with multisystem inflammation after infection, especially in children.	MIS-C may involve shock and cardiac dysfunction; uncommon and distinct from routine acute viral symptoms.	[132]
Long-term postacute condition	Fatigue, post-exertional symptom exacerbation, cognitive problems, dysautonomia, and persistent respiratory complaints.	Long COVID is heterogeneous and may involve functional impairment lasting months or years; assess independently of acute severity.	[12, 13, 202, 209]

ARDS: acute respiratory distress syndrome; ENT: ear, nose, and throat; MIS-C: multisystem inflammatory syndrome in children

Asymptomatic and mild COVID-19

A substantial proportion of SARS-CoV-2 infections are asymptomatic or produce manifestations that do not impair oxygenation. Mild disease commonly presents with sore throat, nasal congestion, cough, malaise, headache, myalgia, low-grade fever, or gastrointestinal complaints. Individual manifestations have limited diagnostic specificity because they overlap extensively with influenza, respiratory syncytial virus infection, and other viral respiratory syndromes. Confirmation, when clinically necessary, therefore depends on appropriate virological testing rather than symptoms alone [116,117].

Chemosensory dysfunction was a distinctive feature during early transmission periods. A European multicentre study of laboratory-confirmed mild-to-moderate cases described olfactory and gustatory complaints, frequently occurring without severe respiratory symptoms. Although anosmia and dysgeusia can still be encountered, their diagnostic utility varies with circulating lineages and host characteristics and should not be considered obligatory manifestations [120]. Headache, dizziness, reduced appetite and transient gastrointestinal symptoms may occur either alongside respiratory complaints or as prominent presenting symptoms.

An important clinical distinction exists between mild symptoms and low risk of complications. People of advanced age, severe immunosuppression, substantial cardiopulmonary disease, or multiple comorbidities may initially report only minor symptoms while remaining susceptible to deterioration. At the same time, routine hospital referral of all infected individuals is unnecessary; most immunocompetent patients with uncomplicated illness recover with appropriate symptom management and monitoring. Factors informing escalation include new breathlessness, persistent chest discomfort, altered cognition, dehydration, worsening functional status and objective evidence of impaired oxygenation [24,116,119].

The frequency of asymptomatic infection is difficult to quantify consistently because screening intensity, testing intervals, symptom questionnaires and follow-up duration vary across investigations. Some apparently asymptomatic cases are subsequently reclassified as presymptomatic, whereas very mild complaints may be unrecognized or recalled inconsistently. Consequently, numerical comparisons between studies should be interpreted with particular caution when ascertainment and immune backgrounds differ [68,69].

Moderate, severe and critical COVID-19

Lower respiratory tract involvement defines an important transition in the clinical spectrum. Patients with moderate illness may have clinical or radiographic evidence of pneumonia without features of severe hypoxemia, although operational definitions vary among guidelines. Severe disease is characterized by clinically significant oxygenation impairment or respiratory distress, while critical disease includes respiratory failure requiring advanced respiratory support, shock, or dysfunction of additional organs. Because definitions and thresholds for admission have changed over time, published proportions assigned to these categories are not directly interchangeable [117,121].

Progressive viral pneumonia typically manifests with increasing cough, exertional or resting dyspnoea, tachypnoea and reduced peripheral oxygen saturation. Fever may be absent in older adults or immunocompromised individuals, and subjective breathlessness may underestimate physiological impairment. Some patients exhibit marked hypoxemia without proportionate respiratory discomfort, a phenomenon often termed silent or happy hypoxemia; clinical interpretation nevertheless requires attention to respiratory effort, measurement limitations and the overall trajectory rather than a distinctive disease phenotype [116,121].

Intensive care cohorts from the initial pandemic waves documented high requirements for invasive mechanical ventilation, frequent ARDS and considerable morbidity. In a Lombardy regional series involving 1,591 intensive care unit patients, respiratory support was required for nearly all individuals with available support data, reflecting both the seriousness of the first-wave hospitalized population and the prevailing patterns of care. These historical findings should not be extrapolated to contemporary infection-fatality or admission risks [121].

Clinical deterioration may reflect diffuse inflammatory alveolar injury, pulmonary vascular abnormalities, bacterial or fungal superinfection, cardiogenic components of pulmonary oedema, or combinations of these processes. Although COVID-19-associated ARDS can exhibit heterogeneous lung compliance and perfusion abnormalities, available evidence has not

established a universally distinct ARDS entity that warrants abandoning established physiological principles of assessment and ventilatory management [106,122]. Critical illness is further complicated by shock, acute kidney injury, delirium, myocardial injury and thromboembolism, with risks influenced by the timing and intensity of supportive interventions [118,119].

Respiratory manifestations and complications

The respiratory system is the predominant site of clinically important acute disease. Upper airway symptoms may be followed by bronchitic illness, viral pneumonitis or diffuse pulmonary involvement. Typical chest imaging abnormalities during severe ancestral-virus infection included bilateral ground-glass opacities, patchy consolidation and peripheral or lower-zone predominance, although neither the distribution nor the pattern is pathognomonic. Imaging should be interpreted together with clinical probability, virological results and alternative diagnoses; routine computed tomography (CT) is not required in otherwise uncomplicated infection [2,116].

In patients progressing to acute hypoxemic respiratory failure, gas-exchange abnormalities arise from ventilation–perfusion mismatch, alveolar flooding, microvascular injury and loss of aerated lung units. Respiratory mechanics vary substantially between patients and across disease phases. Severe dyspnoea, accessory muscle use, increasing oxygen requirements and deteriorating arterial oxygenation are clinically meaningful manifestations, but oxygen saturation may be affected by perfusion and device-related error. Thus, a single peripheral oxygen saturation reading should not be considered sufficient to characterize the severity of respiratory compromise [106,108,122].

ARDS represents one of the most consequential acute complications and can coexist with pulmonary embolism, ventilator-associated complications or secondary infection. Histopathological investigations described endothelial injury and thrombotic microangiopathy, supporting an interaction between inflammatory alveolar damage and pulmonary vascular pathology. These mechanisms may contribute to disproportionate hypoxemia in selected patients but do not imply that all lung injury is directly mediated by viral cytopathic activity [106,108].

Spontaneous pneumomediastinum, pneumothorax and organizing pneumonia have also been reported during or after acute illness, particularly in selected hospitalized cohorts. However, complications should be attributed cautiously: mechanical ventilation, positive airway pressure, underlying lung disease and prolonged inflammatory injury are potential contributors. Persistent breathlessness after the acute phase requires a distinct diagnostic approach and will be considered in the dedicated long COVID section rather than conflated with active viral pneumonia [12,13].

Cardiovascular manifestations and complications

Acute SARS-CoV-2 infection is associated with a range of cardiovascular abnormalities, encompassing myocardial injury, arrhythmias, heart failure, acute coronary syndromes, venous and arterial thromboembolic events, and circulatory shock. Elevated cardiac troponin concentrations are repeatedly associated with an adverse prognosis among hospitalized patients. Nevertheless, biochemical myocardial injury is a syndrome-level observation rather than a diagnosis of viral myocarditis: potential causes include oxygen supply–demand imbalance, sepsis, microvascular dysfunction, pulmonary embolism and pre-existing cardiovascular disease [119,123].

An early Wuhan cohort identified an association between cardiac troponin elevation, underlying cardiovascular disorders and fatal outcomes. This observation established prognostic relevance but did not determine that direct viral cardiomyocyte infection was the dominant mechanism. Similarly, clinical diagnoses of myocarditis or pericarditis require evaluation based on accepted diagnostic criteria and should not be inferred from nonspecific chest pain, elevated biomarkers, or isolated imaging abnormalities [123].

In severe COVID-19, arrhythmias may be precipitated by systemic inflammation, hypoxemia, electrolyte disturbances, myocardial ischaemia or pharmacological exposures. Right ventricular strain may accompany extensive pulmonary disease or pulmonary thromboembolism. Acute decompensated heart failure can occur in patients with previously reduced reserve as well

as those without recognized structural heart disease. Distinguishing these mechanisms is central to rational management because their treatments and prognostic implications differ [106,108,123].

The observed burden of cardiovascular events is also affected by ascertainment. Hospitalized and critically ill individuals undergo more frequent cardiac biomarker testing and imaging than outpatients, whereas comparative studies may differ in their use of active surveillance and diagnostic thresholds. Accordingly, raw event frequencies should not be interpreted as causal effects of SARS-CoV-2 without an appropriate comparator, time-at-risk definition and adjustment for baseline cardiovascular risk. Longer-term cardiovascular consequences are discussed separately under post-COVID-19 condition [13,123].

Neurological and neuropsychiatric manifestations

Neurological manifestations range from relatively common and nonspecific complaints to uncommon but serious central or peripheral nervous system disorders. Headache, dizziness, altered taste or smell, sleep disturbance and impaired attention may accompany otherwise mild infection. In severe disease, encephalopathy and delirium frequently arise through combined effects of systemic inflammation, hypoxemia, metabolic disturbances, medication exposure and critical illness. The mere detection of neurological symptoms does not establish direct neuroinvasion, and evidence of viral material in neural tissue requires careful interpretation [88,124].

Acute cerebrovascular events, seizures, peripheral neuropathies, acute inflammatory disorders and encephalitis-like syndromes have been described. A systematic review and meta-analysis documented stroke among patients with COVID-19, with important heterogeneity according to study setting and case mix. Possible contributors include conventional vascular risk factors, inflammation-associated thrombosis, atrial arrhythmias and severe systemic illness. Because stroke remains uncommon relative to all SARS-CoV-2 infections, estimates from hospitalized series should not be applied to unselected community infections [124,125].

Neuropsychiatric symptoms may include anxiety, depressive symptoms, agitation, hallucinations and sleep disturbance, but causal attribution can be challenging, particularly when pre-existing conditions, social stressors, intensive care exposure and medications are present. Acute delirium warrants urgent evaluation for reversible causes and should not be automatically categorized as COVID-19-related encephalitis. Similarly, prolonged cognitive symptoms after clearance of acute infection should be differentiated from acute neurological complications and assessed within the framework of post-COVID-19 condition [12,13,124].

The diagnostic approach should be driven by the neurological syndrome and the presence of focal deficits, meningeal signs, seizures or reduced consciousness. Appropriate investigations may include neuroimaging, electroencephalography, cerebrospinal fluid analysis or vascular assessment according to clinical indications. Both under-recognition and over-attribution are potential sources of error; the strongest conclusions are derived from studies using standardized definitions and relevant comparator populations [124,125].

Gastrointestinal, hepatic, renal and endocrine manifestations

Gastrointestinal symptoms are well-recognized components of acute COVID-19. Anorexia, nausea, vomiting, diarrhoea and abdominal discomfort may occur independently of prominent respiratory symptoms. A systematic review incorporating 60 studies and 4,243 patients estimated that 17.6% (95%CI: 12.3–24.5%) experienced gastrointestinal symptoms during the early pandemic; substantial heterogeneity in patient selection and symptom ascertainment limits the applicability of this pooled estimate to contemporary populations [126]. Viral RNA can be detected in fecal specimens, but nucleic acid positivity alone does not demonstrate efficient fecal–oral transmission or active intestinal replication at the time of collection [87,126].

Liver biochemical abnormalities, especially aminotransferase elevations, are frequently observed among hospitalized patients. Their differential diagnosis includes systemic inflammation, hypoxic or ischaemic hepatitis, drug-associated hepatotoxicity, pre-existing liver disease and vascular injury. An isolated biochemical abnormality does not necessarily imply severe hepatic dysfunction, whereas jaundice, impaired synthetic function or rapidly progressive

laboratory changes warrant broader evaluation. Attribution to direct hepatocellular infection should be approached cautiously [87,118].

Acute kidney injury (AKI) may complicate severe COVID-19 and is associated with adverse outcomes. In a large early US hospital cohort, AKI was frequent and was associated with respiratory failure, illustrating the close clinical relationship between pulmonary and renal organ dysfunction. Potential contributors include haemodynamic instability, systemic inflammation, endothelial injury, nephrotoxic medications and underlying chronic kidney disease. Renal impairment should therefore be evaluated using serial creatinine measurements, urine output and context-specific investigation rather than assuming a singular SARS-CoV-2-mediated renal mechanism [127].

Metabolic and endocrine complications include stress hyperglycemia, deterioration of pre-existing diabetes, diabetic ketoacidosis in susceptible patients and alterations in thyroid or adrenal function during critical illness. Interpretation is complex because glucocorticoid exposure, acute inflammatory stress, nutritional changes and previously unrecognized endocrine disorders may contribute. Reviews of the endocrine literature emphasize that both acute infection and its treatment can alter glucose regulation and endocrine test results; these effects should not automatically be equated with permanent glandular injury [128].

Hematological, thromboembolic and other systemic complications

COVID-19-associated coagulopathy includes abnormalities in coagulation biomarkers, platelet activation, endothelial injury and clinically overt thrombosis. Elevated D-dimer is common among hospitalized patients but lacks specificity for venous thromboembolism and may reflect systemic inflammation or nonthrombotic fibrin turnover. Clinical suspicion of pulmonary embolism or deep-vein thrombosis therefore requires integration of symptoms, pretest probability, physiological findings and indicated imaging rather than reliance on an isolated laboratory threshold [107,108].

Both venous and arterial events have been observed during acute illness, particularly in cohorts requiring intensive care. A New York City health-system report documented thrombotic complications among hospitalized patients during the first pandemic wave, although event ascertainment and the competing risk of death influence observed proportions. Mechanistic studies support contributions from endotheliopathy, activated innate immune pathways and immunothrombosis, while immobility, central venous catheters and conventional thrombotic risk factors remain clinically important [106–108,129].

Thrombocytopenia, lymphopenia, changes in fibrinogen and elevated inflammatory indices may accompany severe disease. These findings should be interpreted dynamically and in relation to medications, bacterial superinfection and other conditions that produce overlapping abnormalities. Uncommon severe hematological syndromes require independent diagnostic assessment; not every coagulation disorder occurring in a patient with SARS-CoV-2 infection is caused by the virus itself [95,107,129].

Other systemic complications include shock, secondary bacterial or fungal infection, rhabdomyolysis, pressure injury, delirium and intensive-care-associated weakness. The frequency of these outcomes depends heavily on baseline vulnerability, hospital practices and duration of critical illness. Several complications represent indirect consequences of severe infection, mechanical support or treatment rather than unique manifestations of SARS-CoV-2; this distinction is essential when interpreting clinical outcome studies and estimating attributable disease burden [117,119].

Clinical manifestations in special populations

Children and adolescents

Pediatric SARS-CoV-2 infection is often asymptomatic or mild, with fever, cough and upper respiratory complaints among commonly reported manifestations. An early Chinese pediatric epidemiological investigation and a subsequent synthesis of systematic reviews showed that clinical presentations are heterogeneous and that severe acute disease occurs in a minority of infected children. Nevertheless, low overall pediatric severity does not imply uniform risk across age groups or underlying health conditions [130,131]. A rare postinfectious hyperinflammatory

syndrome, multisystem inflammatory syndrome in children (MIS-C), was characterized by fever, systemic inflammation and variable cardiovascular, gastrointestinal and mucocutaneous involvement, often appearing after recent SARS-CoV-2 exposure. MIS-C is clinically distinct from uncomplicated acute infection and must be differentiated from other inflammatory and infectious syndromes [132]. In a US surveillance cohort encompassing hospitalizations during 2022–2024, underlying chronic lung, cardiovascular, neurological and metabolic disorders were associated with higher risk of severe disease in relevant pediatric age strata; these later data illustrate that vulnerable children remain at risk despite changing population immunity [133].

Older adults

Advanced age remains one of the strongest determinants of poor outcome, especially in individuals with frailty, cognitive impairment, or multimorbidity. Conventional symptoms may be absent or less prominent, whereas falls, functional decline, reduced oral intake, confusion or delirium may dominate presentation. An observational study of hospitalized older adults identified atypical initial complaints in a substantial proportion of patients, reinforcing the importance of considering SARS-CoV-2 infection when abrupt functional or cognitive deterioration accompanies community respiratory virus circulation [24,134].

Pregnancy and the neonatal period

COVID-19 during pregnancy poses clinically important risks that vary with gestational age, maternal comorbidities, variant period and vaccination status. A living systematic review identified associations between maternal COVID-19 and selected adverse maternal and perinatal outcomes. These findings require interpretation in light of differences in testing strategies, obstetric populations, and comparator definitions; not every adverse obstetric event is directly caused by SARS-CoV-2. Clinical assessment should distinguish disease severity in the pregnant patient from obstetric indications for admission or delivery [135].

Immunocompromised individuals and patients with chronic disease

Solid-organ transplant recipients, people with hematological malignancies, and patients receiving potent immunosuppressive therapies may experience atypical symptoms, delayed viral clearance, or prolonged replication. A systematic review across immunocompromised groups emphasized the heterogeneity of clinical risks according to underlying condition and treatment. Patients with active cancer were also shown in a multinational cohort to have clinically important adverse outcomes, although cancer stage, functional status and treatment exposures complicated interpretation. Clinical risk cannot be defined by a single label such as 'immunocompromised'; the type and degree of immune dysfunction are central to assessment [136,137].

Clinical characteristics associated with contemporary SARS-CoV-2 variants

The clinical phenotype of COVID-19 has changed as viral evolution occurred in populations with increasing vaccination coverage, infection-derived immunity and access to treatment. Omicron emergence was associated with lower observed risks of severe outcomes than Delta in several populations, but the magnitude of the difference depended on host immune history and the endpoint examined. In a large English cohort, Omicron infection was associated with a lower adjusted hazard of hospital admission and death than Delta infection, even though substantial clinical risk persisted among vulnerable patients [138]. These findings should not be interpreted as proof of a universal evolutionary trajectory toward attenuation.

Southeast Asian cohorts provide important evidence that the variant-era pattern observed in high-income countries is not necessarily generalizable across healthcare settings. An Indonesian multicentre prospective cohort spanning pre-Delta, Delta and Omicron periods observed the highest age-adjusted severe-disease proportion during Delta predominance and a lower proportion during Omicron predominance. Differences in ascertainment, treatment, vaccination and available care complicate comparisons across these periods, and hospital-based samples do not estimate population-wide infection severity [139].

Within Omicron, divergent results have been reported for specific sublineages. An Indonesian hospital-based analysis associated BA.5 sublineages with higher adjusted odds of severe symptoms relative to BA.1/BA.2 among enrolled patients, whereas a South Korean

multicentre cohort identified lower adjusted odds of severe pneumonia and several poor clinical outcomes during BA.5-predominant periods than during BA.1/BA.2-predominant periods. These results are not necessarily contradictory: study design, lineage assignment, hospitalization selection, and the distribution of previous immunity differ, highlighting the limitations of assigning intrinsic pathogenicity from observational comparisons alone [140,141].

For more recently circulating descendants, changes in receptor affinity, neutralization sensitivity or experimental replicative fitness should not be used as direct substitutes for clinical severity data. Valid attribution requires high-quality patient-level studies with reliable lineage identification, adjustment for prior immunity and comorbidities, standardized severity definitions and sufficient follow-up. Until such data are available, strong claims that an emerging lineage causes milder or more severe disease systematically should be avoided [48,50-53].

Predictors of disease progression, hospitalization and mortality

Risk prediction in COVID-19 requires differentiation between susceptibility to infection, progression after confirmed infection, hospital admission and fatal outcome. Advanced age, frailty, multimorbidity, obesity, diabetes, cardiovascular and chronic pulmonary disease, chronic kidney disease and clinically meaningful immunosuppression have been repeatedly associated with poor outcomes. Yet associations estimated among hospitalized patients are conditioned on referral and admission practices and can differ from the risks observed in community-based populations [24,117,119].

A prospective cohort of more than 5,000 individuals in New York identified clinical and demographic characteristics associated with admission and critical illness, including oxygenation abnormalities and measures of baseline clinical vulnerability. More broadly, national risk models such as QCOVID combined demographic characteristics and chronic diseases to estimate serious COVID-19 outcomes, demonstrating the value of multivariable assessment over single-factor thresholds. However, calibration may deteriorate when a model is transported to populations with different circulating lineages, vaccination status, care availability, or outcome definitions [142,143].

Sex differences also warrant careful interpretation. A large systematic review and meta-analysis identified male sex as a risk factor for intensive treatment unit admission and death, although the underlying explanations likely include biological, behavioral and health-system factors and cannot be established through pooled associations alone. In contrast, the presence of a chronic disorder is not interchangeable with its severity: uncontrolled diabetes, decompensated cardiovascular disease and profound immunosuppression may confer different risks than stable, well-managed conditions within the same diagnostic categories [24,144].

Objective clinical assessment remains essential. Worsening oxygenation, increasing respiratory rate, hypotension, new encephalopathy, and evolving dysfunction of other organs indicate immediate clinical concern. Among hospitalized patients, abnormalities in lymphocyte count, inflammatory biomarkers, renal function, cardiac troponin and coagulation indices can support risk assessment, but none should be treated as a stand-alone determinant of prognosis. Prognostic algorithms must be judged by external validation, calibration, clinical usefulness and the potential for bias rather than solely by discrimination statistics [118,119,142,143].

In 2026, the most defensible clinical model of risk is dynamic and context-dependent: it accounts for clinical trajectory, individual immune history, the timing of testing and treatment, local healthcare resources and the differential diagnosis. This approach recognizes that the overall distribution of COVID-19 severity has changed substantially since 2020 while maintaining vigilance for severe disease in populations with reduced physiological or immunological reserve [21,24,112,133,139].

Diagnosis of COVID-19

Clinical assessment and diagnostic considerations

The diagnosis of COVID-19 requires integration of clinical probability, epidemiological context, and direct detection of SARS-CoV-2. Although the initial pandemic response emphasized identification of nearly all suspected infections, contemporary diagnostic decisions are increasingly individualized according to clinical severity, the likelihood of alternative respiratory

infections, vulnerability to progression and whether confirmation would alter antiviral treatment, infection-control measures or patient disposition. Fever, cough, sore throat, nasal congestion, myalgia and fatigue are compatible with COVID-19 but lack sufficient specificity to differentiate SARS-CoV-2 infection from other viral respiratory syndromes. The absence of fever, particularly in older or immunocompromised patients, should not be interpreted as evidence against infection. Accordingly, symptom-based screening alone cannot establish or reliably exclude COVID-19 [145].

The pretest probability of infection is influenced by recent exposure, the timing of symptom onset, local SARS-CoV-2 circulation, prior infection, vaccination history and the presence of competing diagnoses. Vaccination and pre-existing immunity modify the probability of severe disease but do not eliminate infection; conversely, a positive molecular result may identify incidental SARS-CoV-2 detection in a patient whose principal acute illness has another cause. A clinically coherent diagnostic pathway therefore distinguishes three questions: whether current SARS-CoV-2 infection is present, whether the infection accounts for the observed syndrome, and whether the patient is at risk of progression or needs an intervention. The evidentiary threshold differs for each question. Particularly among hospital admissions, interpretation should incorporate symptom chronology, oxygenation, examination findings, comorbidities and the anticipated clinical consequences of diagnostic uncertainty [145].

Decisions regarding testing also depend on health-system constraints. In settings with limited access to centralized molecular laboratories, timely availability of a somewhat less sensitive test may be more useful for immediate triage than a reference assay whose result arrives after the treatment window. Nevertheless, diagnostic decentralization should not be conflated with equivalence between test modalities. Quality-assured specimen collection, clear laboratory reporting and an explicit strategy for managing discordant findings remain central to a clinically effective approach. Repeated testing should be reserved for situations in which a negative initial result is inconsistent with a sufficiently high clinical suspicion or would otherwise change management [145]. The principal diagnostic modalities, their applications, and their interpretive limitations are presented in **Table 4**.

Nucleic acid amplification tests and reverse transcription polymerase chain reaction

Nucleic acid amplification tests (NAATs), particularly real-time RT-PCR, remain the principal laboratory reference methods for detecting acute SARS-CoV-2 infection. Following viral RNA extraction, reverse transcription generates complementary DNA, which is amplified through sequence-specific primers and probes. This diagnostic workflow, from specimen processing to real-time amplification and signal detection, is illustrated in **Figure 4**. Assays generally target conserved regions within the nucleocapsid, envelope, ORF1ab or other viral genomic regions, with multi-target designs providing partial protection against assay failure caused by sequence variation. The early development of validated RT-PCR workflows enabled rapid dissemination of diagnostic capability across clinical and public health laboratories. Contemporary systems include high-throughput centralized platforms and rapid cartridge-based NAATs, whose analytical performance, turnaround time and laboratory requirements differ [145,146].

A negative NAAT result does not independently exclude infection because clinical sensitivity is conditional on sampling time and quality. In a pooled analysis from the early pandemic, Kucirka *et al*. estimated marked variation in the false-negative probability across the interval between exposure and symptom onset; this finding remains conceptually important, although numerical estimates derived from early strains and sampling protocols should not be transferred uncritically to the Omicron era [147]. Upper respiratory viral burden may also fluctuate within an individual, while inadequate collection, transport delays, sample inhibitors and failure to sample an anatomically relevant site can compromise detection. A high-quality specimen obtained after symptom onset may be more informative than a poorly timed test obtained immediately after exposure [147].

Nasopharyngeal specimens were widely used during the initial pandemic, but saliva, midturbinate and anterior nasal specimens can reduce discomfort and enable self-collection. A systematic review comparing saliva and nasopharyngeal-swab NAATs found broadly comparable diagnostic performance in appropriately designed studies, although heterogeneity in specimen

processing and collection procedures affected pooled estimates. The preferred specimen is therefore determined by assay validation, disease stage, feasibility and the clinical question. In patients with severe lower respiratory tract disease and persistently negative upper respiratory samples, a lower respiratory specimen may be considered when the expected incremental diagnostic yield justifies the procedure and its risks [145,148].

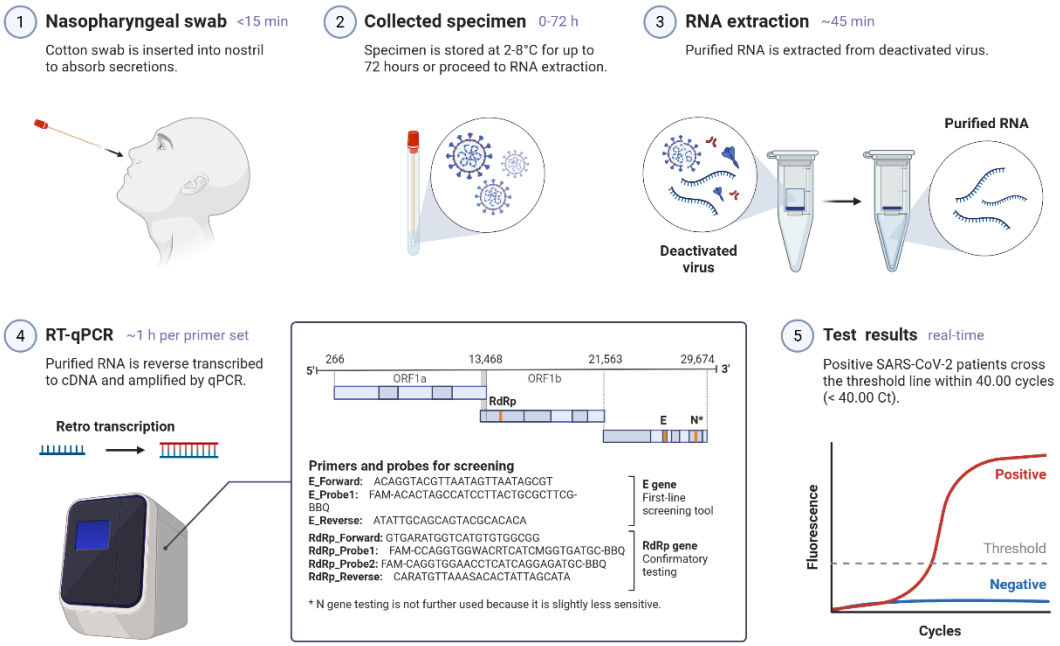


Figure 4. Detection of SARS-CoV-2 by real-time reverse transcription polymerase chain reaction (RT-PCR). The workflow involves specimen collection, viral RNA extraction, reverse transcription to complementary DNA, and amplification of SARS-CoV-2 genomic targets using sequence-specific primers and probes. Fluorescence is monitored in real time, and signal detection above the assay-specific threshold indicates a positive result. Assays may target conserved regions of the nucleocapsid, envelope, ORF1ab, or other viral genes, depending on the platform used.

Cycle-threshold (Ct) values require particularly cautious interpretation. Ct represents the number of amplification cycles necessary to cross a predetermined detection threshold and is influenced by assay chemistry, specimen type, extraction efficiency and platform calibration. Although lower Ct values often correlate with greater RNA abundance within the same validated system, Ct is not interchangeable with an absolute viral load and does not establish the presence of replication-competent virus. Virus isolation studies illustrate that the probability of recovering infectious virus diminishes as RNA concentrations decline, but relationships vary according to host factors, time since infection and laboratory methods. Consequently, a single Ct value should not be used as a universal criterion for contagiousness, severity or termination of precautions [149].

Rapid antigen detection tests

Rapid antigen diagnostic tests identify viral proteins, most commonly the nucleocapsid antigen, through lateral-flow immunoassays or other immunochemical detection formats. Their principal advantages are short turnaround time, minimal equipment requirements, relatively low cost and availability for home or community use. However, antigen tests are inherently affected by the abundance of target protein at the sampled site and demonstrate lower average clinical sensitivity than well-performed NAATs. In the 2025 Cochrane update focusing on asymptomatic populations, Dinnes *et al*. documented substantial variation across brands and settings and generally low sensitivity in population-screening contexts, despite consistently high specificity [150]. Accordingly, an antigen result must be interpreted in relation to symptoms, time since exposure, background prevalence and assay-specific validation [150].

Positive rapid antigen results obtained with high-specificity assays are generally clinically actionable when pretest probability is not extremely low, whereas a negative result does not exclude early or low-burden infection. The Infectious Diseases Society of America (IDSA) guideline emphasizes the dependence of test sensitivity on symptom status and timing and recognizes antigen testing as a useful alternative when molecular testing is inaccessible or delayed. For patients in whom antiviral treatment or isolation decisions depend on accurate confirmation, molecular testing is preferable when a single negative antigen result conflicts with the clinical assessment. The decision to repeat antigen testing should follow the manufacturer's validated instructions and the relevant clinical guidance rather than an assumption that any arbitrary repetition strategy will produce equivalent performance [151].

The relationship between antigen positivity and infectiousness is informative but incomplete. In a prospective household cohort, home antigen testing demonstrated closer correspondence with same-day viral culture than with RT-PCR, although sensitivity remained imperfect and changed across the course of illness. These findings are biologically plausible because antigen detection tends to require greater viral abundance than amplification-based methods, but antigen positivity must not be treated as a direct measure of transmissibility. Conversely, persistent RT-PCR positivity in the absence of a positive antigen result may reflect residual RNA rather than active infection; this pattern is particularly sensitive to the timing of sampling. Serial results are therefore best interpreted as evolving probabilistic evidence rather than a definitive declaration that an individual is or is not infectious [152].

Serological assays and their clinical applications

Serological assays identify host antibodies directed against SARS-CoV-2 antigens, principally spike or nucleocapsid proteins, and should be distinguished from tests designed to diagnose current viral replication. Immunoglobulin G, immunoglobulin M and total-antibody assays may be performed using enzyme-linked immunosorbent, chemiluminescence or other immunoassay technologies. The principle of antibody detection using a lateral flow assay is illustrated in **Figure 5**. Because antibody responses require time to develop, serological tests are insufficiently sensitive during the early stages of acute illness and are not recommended as standalone tools for diagnosing recent infection. Updated Infectious Diseases Society of America recommendations advise against routine serology for diagnosis within the first two weeks of symptom onset and recognize only limited circumstances in which antibody testing contributes to establishing prior infection [153].

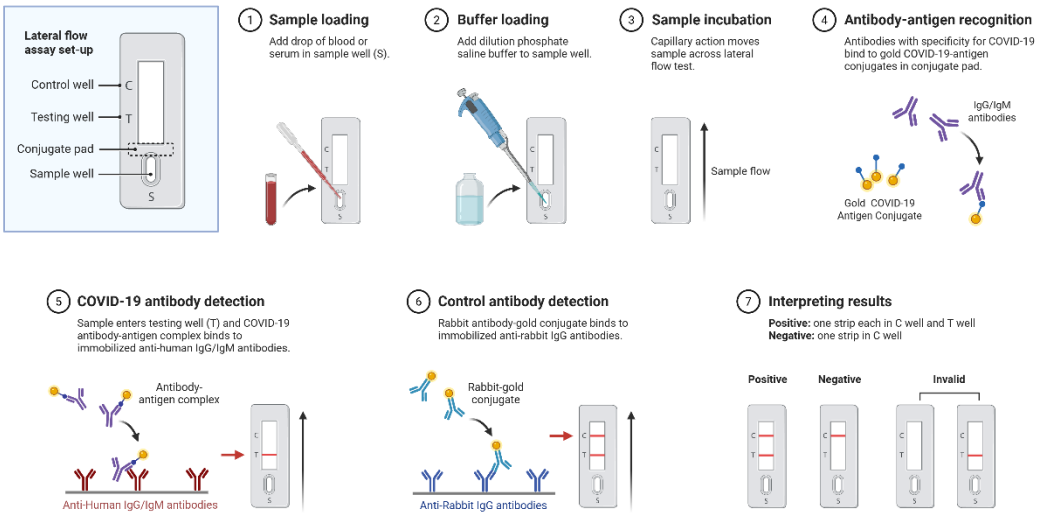


Figure 5. Principle of SARS-CoV-2 antibody detection using a lateral flow assay. The assay detects SARS-CoV-2-specific immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies in blood through antigen–antibody interactions. Visible test and control lines indicate the result, with the control line confirming test validity. Serological assays detect antibody responses to infection or vaccination but cannot independently confirm active SARS-CoV-2 infection.

Table 4. Diagnostic modalities for SARS-CoV-2 infection: Principles, applications, and limitations

Modality	Biological principle or specimen	Primary applications and strengths	Main limitations and interpretive cautions	References
Laboratory RT-PCR/NAAT	Amplification of SARS-CoV-2 RNA from upper or lower respiratory specimens.	Reference method for confirmation; typically high analytic sensitivity; useful for high-risk patients and hospital pathways.	Negative results depend on collection, timing, specimen quality and assay targets; positive RNA detection does not necessarily imply infectious virus.	[145–148]
Rapid point-of-care molecular NAAT	Cartridge-based PCR or isothermal nucleic-acid amplification at or near the patient.	Faster triage with molecular detection; valuable where immediate antiviral decisions are needed.	Platform-specific sensitivity, quality controls, availability and cost; low viral load can affect performance.	[145, 166]
Rapid antigen test	Lateral-flow or instrument-assisted detection of viral proteins, often nucleocapsid antigen.	Accessible, inexpensive and rapid detection, especially during high viral burden.	Lower sensitivity than NAAT, especially with early/late infection or asymptomatic testing; negative results do not exclude infection.	[150–152]
Serial antigen testing	Repeated antigen tests after an initial negative result, according to clinical context and test instructions.	May identify infections missed during early low-antigen phases.	Interpretation depends on pretest probability and test interval; serial testing does not provide universal exclusion.	[150–152]
Multiplex respiratory NAAT	Concurrent nucleic-acid detection of SARS-CoV-2, influenza viruses, RSV, and other pathogens.	Useful for syndromic assessment when co-circulation affects isolation and treatment decisions.	Co-detection may occur; test panel composition, specimen timing and interpretive clinical context remain important.	[162–164]
Serology: anti-N/anti-S	Measurement of infection- or vaccine-associated antibody responses in blood.	Supports selected retrospective, seroepidemiological, or immunological investigations.	Not recommended as a stand-alone test for acute infection or a reliable individual immune-protection threshold; anti-S may reflect vaccination.	[153, 154]
Whole-genome sequencing	Sequencing of viral genetic material from suitable positive samples.	Variant identification, outbreak investigation, transmission reconstruction and evolutionary surveillance.	Turnaround time, cost, coverage, low viral loads and nonrepresentative sampling limit routine clinical use.	[55, 155, 270]
Viral culture	Propagation of replication-competent virus in permissive cell systems.	Research on infectiousness, antiviral susceptibility, and virus biology.	Specialized biosafety requirements; limited clinical availability; negative culture does not prove noninfectiousness.	[149, 152]
Chest radiography/CT	Imaging of pulmonary parenchymal changes and complications.	Assesses extent of pneumonia, hypoxemia complications, or alternate diagnoses in selected patients.	Ground-glass opacities and other findings are nonspecific; imaging cannot confirm SARS-CoV-2 infection.	[159–161]
Inflammatory and organ-injury biomarkers	Blood count, CRP, D-dimer, troponin, creatinine and other context-specific tests.	Support severity assessment, organ monitoring and differential diagnosis, especially in hospitalized patients.	None reliably establishes the viral etiology; thresholds vary with age, comorbidity, disease stage and care setting.	[118, 158]
Emerging CRISPR/other isothermal tests	Guide-RNA-based recognition or rapid amplification coupled to fluorescent or lateral-flow readouts.	Potential for decentralized, rapid molecular testing and multiplex assay development.	Assay-to-assay heterogeneity, validation, regulatory clearance, and real-world accuracy remain key barriers.	[165–168]

CRISPR: clustered regularly interspaced short palindromic repeats; CRP: C-reactive protein; CT: computed tomography; NAAT: nucleic acid amplification test; RT-PCR: reverse transcription polymerase chain reaction; RSV: respiratory syncytial virus

Antigen selection determines the interpretation of a positive antibody test. Spike-specific antibodies may arise following infection or vaccination with spike-encoding formulations, whereas nucleocapsid antibodies can provide evidence of previous infection among recipients of spike-only vaccines. This distinction is not universal: inactivated whole-virus vaccine recipients may also develop responses to nucleocapsid, while nucleocapsid seroreversion and variable assay sensitivity can obscure remote infections. Distinct serological patterns after natural infection and mRNA vaccination have been demonstrated empirically, but these patterns cannot be generalized indiscriminately across vaccine platforms, previous exposure histories or all commercial assays [154].

Serology remains valuable for population serosurveys, characterization of immune responses, selected investigations of postinfectious inflammatory syndromes and carefully defined clinical questions in immunocompromised populations. However, measured binding-antibody concentrations should not be interpreted as a universal threshold for protection from infection or severe disease. Protection depends on multiple components of immunity, circulating variants, exposure intensity and host characteristics, and antibody assays differ in their antigenic targets and calibration. Standardization against recognized reference materials improves comparability but cannot eliminate biological differences between assays. Therefore, routine clinical decisions concerning vaccination or antiviral treatment should not be based solely on an isolated quantitative antibody measurement [153,154].

Viral culture and genomic sequencing

Viral culture permits investigation of replication-competent SARS-CoV-2 but has a limited role in routine clinical diagnosis because it requires specialized biosafety infrastructure, viable specimen handling, technically demanding procedures and a substantially longer reporting interval than most molecular assays. Its value is primarily research-oriented, including the assessment of infectious-virus recovery, experimental characterization of variants and evaluation of antiviral susceptibility. A negative culture cannot be interpreted as an absolute absence of infectiousness, given dependence on the specimen and culture system. The continued detection of RNA after viable virus becomes difficult to recover reinforces the distinction between molecular positivity and active infection, although no single culture-derived Ct threshold is transferable across settings [149].

WGS provides substantially different information from routine diagnostic amplification. By characterizing genome-wide substitutions, deletions and recombination patterns, it permits lineage assignment, phylogenetic reconstruction and analysis of potential transmission networks. Its value is particularly evident in suspected healthcare-associated clusters, persistent infection in immunocompromised patients and surveillance of variant emergence. An Indonesian investigation involving immunocompromised children used WGS alongside clinical and epidemiological data to identify strong genetic similarity among hospital-associated cases. Such findings support transmission hypotheses but do not, in isolation, demonstrate direct person-to-person transmission because unsampled intermediates and common exposures remain plausible [155].

Mutation-specific RT-PCR assays and patterns of target failure can function as rapid screening markers for particular variants, but they are neither comprehensive substitutes for sequencing nor stable lineage identifiers across successive evolutionary waves. Hilti *et al.* demonstrated the utility of S-gene target failure as a surveillance indicator in Switzerland and Liechtenstein, while also documenting its dependence on assay characteristics and the variant distribution under study [156]. Recent investigations have extended the public health application of sequencing to residual self-collected antigen-test specimens, demonstrating the possibility of integrating decentralized specimen acquisition with molecular surveillance. This approach remains sensitive to RNA quantity, sampling biases and laboratory logistics and requires quality assurance before population-level inference [156,157].

Laboratory biomarkers and assessment of disease severity

Routine laboratory investigations are most appropriately used to assess disease severity, detect complications and identify alternative or concurrent diagnoses rather than establish the etiology of acute respiratory symptoms. In hospitalized patients, complete blood counts, C-reactive

protein, renal and liver function tests, serum electrolytes, coagulation parameters and markers of tissue injury may support risk stratification or serial assessment. Lymphopenia, elevated inflammatory markers, impaired oxygenation and abnormalities in renal or hepatic indices were frequently observed in early severe-disease cohorts. Nevertheless, these findings are neither specific to SARS-CoV-2 nor consistent across patient populations. Comorbid illness, bacterial coinfection, treatment effects and the timing of measurement may substantially alter their interpretation [142].

Biomarker research has extended beyond conventional acute-phase reactants. A 2025 systematic review and meta-analysis identified potentially informative prognostic associations involving mid-regional pro-adrenomedullin, the neutrophil-to-lymphocyte ratio and Krebs von den Lungen-6. The authors also noted heterogeneous study designs, limited validation and variability in diagnostic thresholds. These biomarkers should therefore be regarded as adjuncts to clinical risk assessment rather than independently validated tools for determining escalation of care. Importantly, discrimination of severe versus nonsevere disease in retrospective datasets does not demonstrate that biomarker-guided management improves survival, reduces admission rates or adds value beyond established clinical prediction models [158].

The prognostic contribution of a laboratory variable also changes with baseline risk. In vaccinated populations and during later variant periods, inflammatory trajectories observed in early unvaccinated inpatient cohorts may overestimate the expected abnormality among patients presenting in contemporary outpatient practice. Interpretation should account for pre-existing kidney disease, immunosuppression, pregnancy and other causes of abnormal laboratory results. Repeated biomarkers are justified when they inform specific decisions, such as evaluating progressive organ dysfunction or a new thromboembolic or bacterial complication. Widespread testing without a management implication may instead generate false alarms, diagnostic cascades and unnecessary costs. As with any diagnostic strategy, incremental clinical utility is more important than statistical association alone [142,158].

Radiological findings and imaging modalities

Chest imaging is important for characterizing pulmonary involvement and detecting complications but cannot replace virological testing for confirmation of SARS-CoV-2 infection. The primary modalities are chest radiography, CT and, where appropriate expertise is available, point-of-care lung ultrasonography. Radiographs may demonstrate bilateral or peripheral opacities in patients with pneumonia, but early or mild infection can produce normal findings. CT may reveal ground-glass opacities, consolidation, or organizing-pneumonia patterns, none of which is pathognomonic for COVID-19. A multinational Fleischner Society consensus emphasized that imaging decisions should be contingent on illness severity, the probability of infection, expected changes in management, and the availability of resources [159].

The diagnostic specificity of CT abnormalities is influenced by background prevalence and competing causes of pulmonary inflammation. In a systematic review and meta-analysis of standardized CT criteria, Nam *et al*. estimated pooled sensitivity of 70% (95%CI: 65%–74%) and specificity of 90% (95%CI: 86%–93%), with substantial heterogeneity [160]. Such summary values underscore why the performance of CT cannot be generalized across stages of illness, study populations, and radiological classification systems. A typical CT pattern may strengthen the probability of COVID-19 in the appropriate context, but findings may also reflect other viral pneumonia, inflammatory lung disease, edema, or pre-existing pathology. Thus, CT should be interpreted as anatomical evidence within a diagnostic pathway rather than as a virological reference test [160].

Imaging also contributes to assessment of complications and prognosis. The extent of pulmonary abnormalities on CT has been associated with risk of severe disease and mortality, although scoring systems differ in calibration and validation. A systematic review by Prakash *et al*. found that CT severity scores showed useful discrimination for clinical severity and mortality in studies largely originating from earlier pandemic periods [161]. These results should not be construed as support for routine CT in all infected patients or as evidence that a score independently determines treatment. Chest radiography or lung ultrasonography may provide sufficient information in selected settings, while CT pulmonary angiography should be reserved

for a specific suspicion of pulmonary embolism rather than performed solely because of SARS-CoV-2 infection [161].

Differential diagnosis with other respiratory infections

The differential diagnosis of COVID-19 has become increasingly important as SARS-CoV-2 co-circulates with influenza viruses, respiratory syncytial virus (RSV), rhinoviruses and other respiratory pathogens. These agents overlap in their principal symptoms, including cough, fever, nasal symptoms, dyspnea, and systemic manifestations, and clinical features rarely provide sufficient discrimination to identify a pathogen with confidence. Distinguishing among them matters because recommended antivirals, eligibility criteria, infection-control decisions and the evaluation of vulnerable patients differ. Multiplex molecular assays that simultaneously detect SARS-CoV-2, influenza A and B, and RSV are therefore increasingly relevant in hospital and selected ambulatory settings, particularly when results can be returned within a therapeutically useful interval [162].

Coinfection introduces another interpretive challenge. Detection of SARS-CoV-2 does not exclude influenza, RSV, or a bacterial cause of the presenting syndrome, and the simultaneous identification of two pathogens does not necessarily establish their relative contribution to disease. A 2024 study describing respiratory virus coinfections across successive SARS-CoV-2 variant periods reinforced the heterogeneity of coinfecting pathogens and age distributions. Diagnostic algorithms should therefore reflect the patient's clinical presentation rather than terminate reflexively after the first positive result. Patients with focal consolidation, hemodynamic instability, unexpectedly severe illness, or clinical deterioration require consideration of bacterial pneumonia and other nonviral diagnoses despite documented SARS-CoV-2 infection [163].

Multiplex lateral-flow antigen platforms offer a potential decentralized approach to syndromic testing, although their sensitivity depends on the individual target, assay brand, viral burden, and specimen timing. In a study of ambulatory patients with acute respiratory illness, Murphy *et al.* reported generally high specificity but heterogeneous sensitivity for assays detecting influenza, RSV, and SARS-CoV-2 164. Consequently, negative multiplex antigen results should not be considered equivalent to exclusion by an adequately collected molecular panel. The choice between targeted and multiplex testing should be informed by local epidemiology, treatment availability, infection prevention priorities and cost-effectiveness, while avoiding unnecessary testing in clinical circumstances where identification of the pathogen would not alter care [162,164].

Diagnostic challenges associated with emerging SARS-CoV-2 variants

Continued viral evolution presents two related diagnostic challenges: alterations in assay target sequences and changes in the timing or anatomical distribution of detectable viral material. Mutations or deletions within primer- or probe-binding regions may reduce amplification efficiency or cause target dropout, particularly for single-target molecular assays. The appearance of S-gene target failure during Alpha and selected Omicron waves illustrates both this vulnerability and the surveillance value of a recognizable amplification pattern. However, a target failure pattern is lineage-dependent and may lose specificity when genetically distinct lineages acquire or lose similar mutations. Routine clinical assays should therefore prioritize validated multi-target designs and ongoing evaluation against circulating genomic sequences rather than reliance on a single mutation-defined signal [156].

Antigen tests may likewise be affected by changes in antigenic epitopes or target abundance, although effects vary among assays. Performance claims established with historical variants cannot be assumed to apply identically to every descendant lineage, and laboratory cross-reactivity experiments may not predict clinical sensitivity in heterogeneous real-world populations. Variant-related diagnostic comparisons are complicated by changes in vaccination coverage, sampling behavior, symptom onset definitions, and specimen types. It is particularly important not to infer a biological defect in an assay merely from a lower positivity rate during a later epidemiological period. Sound evaluation requires clinical reference testing, sequence-confirmed lineage information when feasible, and comparable sampling protocols across groups [150,151].

Diagnostic surveillance should therefore be embedded within a broader genomic and quality-management framework. Signals that warrant investigation include unexpected amplification failures, discordant multi-target patterns, unexplained clusters of negative antigen tests among NAAT-confirmed cases and sustained changes in local test positivity. When sequencing access is limited, representative sentinel sampling and collaborations between hospitals, laboratories and reference centers may provide a practicable alternative to sequencing every positive specimen. Nevertheless, conclusions regarding the clinical performance of emerging-variant diagnostics must be proportionate to the amount and quality of validation evidence, particularly in settings with low testing intensity and substantial changes in healthcare-seeking behavior [157].

Advances in point-of-care and novel molecular diagnostics

The COVID-19 pandemic stimulated rapid development of decentralized molecular technologies intended to preserve the analytical advantages of nucleic acid detection while shortening turnaround time and reducing laboratory dependence. Reverse-transcription loop-mediated isothermal amplification (RT-LAMP), recombinase-based amplification, and CRISPR-associated nucleic acid recognition have been investigated in diverse clinical formats. In an early proof-of-concept study, Broughton *et al*. described a CRISPR-Cas12 assay incorporating isothermal amplification and lateral-flow visualization [165]. Although its small validation cohort supported technical feasibility, such developmental studies should be distinguished from multicenter prospective evaluations that characterize diagnostic accuracy, usability, batch consistency and error rates in routine clinical practice [165].

Systematic evidence for isothermal amplification has grown, but estimates are dependent on patient selection and the molecular reference standard. A meta-analysis by Pu *et al*. reported high pooled performance for RT-LAMP while identifying sensitivity limitations and the possibility that comparison with an imperfect reference method could inflate apparent agreement [166]. Similarly, a systematic review of CRISPR-based SARS-CoV-2 diagnostics reported favorable pooled accuracy but cautioned that selection bias and methodological heterogeneity could limit generalizability. High analytical sensitivity under controlled laboratory conditions does not establish satisfactory clinical performance in early infection, among asymptomatic population, or under the operational constraints of primary-care facilities [166,167].

The future diagnostic landscape is likely to include compact sample-to-answer molecular platforms, improved multiplex panels, digitally connected community assays and automated surveillance of atypical target signatures. A 2025 study demonstrated the technical feasibility of multiplex recombinase-aided amplification for SARS-CoV-2, influenza A virus and RSV, illustrating efforts to consolidate several clinically important respiratory targets within a rapid assay. Potential advantages for low- and middle-income countries include reduced dependence on centralized laboratories and shorter treatment delays, although capital expenditure, supply-chain robustness, calibration, temperature requirements and external quality assurance remain decisive implementation considerations. Artificial intelligence-assisted interpretation and biosensor-based systems also require prospective assessment against clinically meaningful outcomes rather than reliance on benchmark accuracy alone [168].

An optimal COVID-19 diagnostic framework is not defined by the universal use of the most technologically sophisticated test. Instead, testing should be proportionate to pretest probability, clinical severity, treatment eligibility, and the decision being supported. Molecular assays provide the most robust laboratory confirmation, rapid antigen tests facilitate immediate decentralized decisions, serology addresses selected questions concerning previous exposure, and imaging and biomarkers characterize disease manifestations rather than viral etiology. Genomic sequencing adds population and transmission intelligence, while emerging technologies offer opportunities for integration with routine respiratory surveillance. The principal priority for future research is to determine whether innovative tests improve patient outcomes, equitable access, and timeliness of actionable diagnosis across contemporary SARS-CoV-2 variants [145,150,162,168].

Treatment and clinical management

Principles of COVID-19 management and risk stratification

Clinical management of COVID-19 has evolved from empiric treatment of an unfamiliar viral pneumonia to an evidence-based, severity-stratified approach that separates early viral replication from established inflammatory organ injury. Therapeutic decisions are determined principally by the time since symptom onset, requirement for supplemental oxygen, risk of clinical deterioration, concurrent organ dysfunction, and contraindications to available agents. Adults with uncomplicated infection generally benefit from symptomatic measures and selected early antivirals, whereas patients with hypoxemia require assessment for anti-inflammatory therapy, respiratory support and complications. A positive SARS-CoV-2 test alone is insufficient to justify hospitalization or disease-modifying therapy; the attributable contribution of COVID-19 to an acute illness must be distinguished from incidental detection. Recent evidence-based guidance continues to support this phenotype-specific framework [169,170].

Risk assessment should integrate age, immunocompromised status, cardiopulmonary disease, diabetes, obesity, chronic kidney disease, pregnancy-related considerations, frailty, and the anticipated ability to obtain timely medical review. Previous vaccination and infection modify baseline risk but do not abolish severe disease, particularly among profoundly immunocompromised patients. Decisions also depend on respiratory rate, oxygen saturation, work of breathing, hydration, mental status and signs of systemic complications. Prognostic scores and inflammatory biomarkers may support assessment but should not replace repeated clinical evaluation, especially when a patient deteriorates rapidly. The risk threshold at which an antiviral confers meaningful absolute benefit differs between an unvaccinated patient in 2021 and an immunologically experienced patient in 2026 [169,171].

The evolution of clinical evidence additionally requires careful separation of relative and absolute treatment effects. Reductions in hospitalization observed in highly susceptible trial populations cannot be assumed to yield the same number of prevented events when contemporary baseline hospitalization risk is substantially lower. Conversely, an apparently small absolute benefit may remain clinically important for patients with extreme susceptibility or restricted access to advanced care.

Drug availability, affordability, administration route, and monitoring capacity are also consequential in low- and middle-income settings. Treatment algorithms should be updated as resistance patterns, variant prevalence, and local regulatory authorizations change, rather than mechanically extrapolating early-pandemic protocols [169,171]. The principal therapeutic interventions, their mechanisms of action, evidence-supported indications, and key limitations are summarized in **Table 5**.

Management of asymptomatic and mild COVID-19

Most patients with asymptomatic or mild COVID-19 can be managed outside hospital with advice concerning hydration, rest, antipyretics or analgesics when needed, avoidance of unnecessary antibiotics, and recognition of clinical deterioration. Clinical evaluation should determine whether the patient can maintain oral intake, access healthcare if symptoms worsen, and follow measures to reduce onward transmission.

Older adults and immunocompromised persons may initially describe little dyspnoea despite substantial physiological compromise; therefore, a low threshold for reassessment is appropriate when symptoms progress. Pulse oximetry can assist monitoring when used correctly, but isolated readings must be interpreted alongside device limitations, skin pigmentation, peripheral perfusion and clinical findings. There is no indication for routine systemic corticosteroids in uncomplicated infection without a requirement for supplemental oxygen [170].

Early antiviral treatment is principally intended to prevent progression among individuals at clinically meaningful risk, not to eliminate every mild symptom in low-risk patients. Eligibility requires prompt identification, commonly within five days of symptom onset for oral antivirals, with separate administration windows for intravenous agents.

Table 5. Therapeutic interventions for COVID-19: Mechanisms of action, clinical indications, and supporting evidence

Intervention	Mechanism/approach	Evidence-supported context of use	Major limitations or precautions	References
Nirmatrelvir/ritonavir	SARS-CoV-2 main protease inhibition; ritonavir boosts exposure via CYP3A inhibition.	Early outpatient treatment of eligible patients at risk of progression, after assessment of symptom duration and benefit.	Major drug–drug interactions, renal-dose adjustments and contraindications; absolute benefit depends on baseline risk and immune history.	[10, 169, 172–173, 194]
Remdesivir	Intravenous nucleotide analogue inhibiting viral RNA-dependent RNA polymerase.	Early outpatient treatment in high-risk patients and selected hospitalized patients according to disease severity and guideline pathways.	Intravenous administration; infusion logistics and monitoring; timing relative to viral replication matters.	[169, 173, 174]
Molnupiravir	Oral nucleoside analogue causing errors in viral RNA replication.	Alternative outpatient therapy when preferred options are unsuitable, under applicable local authorization/guidance.	Less compelling evidence for preventing hospitalization in vaccinated populations; pregnancy and reproductive safety considerations.	[169, 175, 176]
Systemic corticosteroids	Glucocorticoid-mediated suppression of harmful inflammation.	Severe/critical COVID-19 requiring supplemental oxygen or ventilatory support; dexamethasone is the best-studied agent.	No routine benefit and possible harm in patients who do not require oxygen; hyperglycemia and secondary infections.	[11]
Interleukin-6 receptor blockade	Tocilizumab or sarilumab suppress IL-6 signaling.	Selected hospitalized patients with severe inflammatory disease, escalating oxygen support, or critical illness, generally with corticosteroids.	Evaluate bacterial/fungal infection, liver enzymes and blood counts; clinical eligibility is context specific.	[178, 179, 182]
JAK inhibition	Baricitinib inhibits JAK1/JAK2-mediated inflammatory signaling.	Selected hospitalized patients with increasing oxygen needs or systemic inflammation, typically in addition to corticosteroids.	Dose adjustment in renal dysfunction; consider infection, thrombosis and other treatment-specific risks.	[180–182]
Supplemental oxygen	Restores adequate oxygen delivery in hypoxemic illness.	Patients with clinically important hypoxemia; escalate support according to physiology and response.	Avoid delayed escalation; target saturation and delivery systems must be individualized.	[170, 183]
High-flow nasal oxygen / CPAP	Noninvasive respiratory support to reduce respiratory effort and improve oxygenation.	Selected patients with acute hypoxemic respiratory failure under careful monitoring and escalation pathways.	Failure requires prompt reassessment; interface tolerance, aerosol mitigation and local expertise affect choice.	[183]
Prone positioning / lung-protective ventilation	Optimizes ventilation–perfusion matching; limits ventilator-induced lung injury when intubated.	Awake proning in suitable monitored patients; established critical-care practices for intubated ARDS.	Do not let awake proning delay intubation when indicated; contraindications and hemodynamic tolerance matter.	[122, 184]
ECMO	Extracorporeal gas exchange for refractory respiratory failure.	Highly selected severe ARDS cases in experienced specialist centers.	Resource intensive; selection, complications and organ failure burden constrain use.	[170, 186]
Anticoagulation	Heparin-based prevention or treatment of venous thromboembolism.	Pharmacologic thromboprophylaxis in eligible hospitalized patients; full-dose treatment for confirmed thrombosis and selected noncritical patients.	Benefit–bleeding balance differs by severity; routine therapeutic dosing is not supported for unselected critically ill patients.	[186–188]

Intervention	Mechanism/approach	Evidence-supported context of use	Major limitations or precautions	References
Antibiotics	Antimicrobial treatment of suspected or proven bacterial disease.	Reserved for clinically indicated bacterial coinfection or secondary infection, with antimicrobial stewardship.	SARS-CoV-2 infection itself is not an indication; unnecessary treatment increases resistance and adverse events.	[185]
Passive antibody-based interventions	Neutralizing antibodies or high-titre convalescent plasma provide exogenous antiviral antibodies.	Potentially relevant only to selected immunocompromised groups or preparations active against circulating lineages, subject to jurisdiction.	Variant susceptibility can change rapidly; historical monoclonal products may lose activity and authorization.	[195, 248]
Investigational agents / extended treatment	Novel protease inhibitors and individualized prolonged or combination antiviral strategies.	Clinical trials or carefully supervised specialist contexts, particularly persistent infection in immunocompromised hosts.	Limited comparative evidence; selection of resistant virus, drug interactions and adverse effects need surveillance.	[189, 190, 194, 264, 265]
Therapies not recommended routinely	Interventions without reproducible clinically meaningful benefit, including hydroxychloroquine or ivermectin for COVID-19.	Not recommended as disease-modifying therapy outside properly designed trials.	Biological plausibility or nonrandomized associations should not substitute for adequately controlled clinical outcome evidence.	[196, 197]

ARDS: acute respiratory distress syndrome; CPAP: continuous positive airway pressure; CYP3A: cytochrome P450 3A; ECMO: extracorporeal membrane oxygenation; IL-6: interleukin 6; JAK: janus kinase

The 2026 American College of Physicians practice points emphasize consideration of nirmatrelvir/ritonavir for appropriate high-risk symptomatic outpatients and provide a conditional role for molnupiravir while discouraging ivermectin and variant-inactive antibody products. The 2026 Infectious Diseases Society of America focused update similarly addresses nirmatrelvir/ritonavir, remdesivir and molnupiravir according to individualized anticipated benefit and certainty of evidence [171,169].

Early antiviral treatment is principally intended to prevent progression among individuals at clinically meaningful risk, not to eliminate every mild symptom in low-risk patients. Eligibility requires prompt identification, commonly within five days of symptom onset for oral antivirals, with separate administration windows for intravenous agents. The 2026 American College of Physicians practice points emphasize consideration of nirmatrelvir/ritonavir for appropriate high-risk symptomatic outpatients and provide a conditional role for molnupiravir while discouraging ivermectin and variant-inactive antibody products. The 2026 Infectious Diseases Society of America focused update similarly addresses nirmatrelvir/ritonavir, remdesivir and molnupiravir according to individualized anticipated benefit and certainty of evidence [171,169].

Patient education should explain that therapeutic eligibility cannot be inferred solely from a positive test, that early symptom improvement does not exclude later deterioration, and that symptoms sometimes recur after apparent recovery. Evidence that early antiviral therapy prevents post-COVID-19 condition remains less definitive than evidence concerning acute hospitalization or death. Shared clinical decisions are especially important when a patient is taking interacting medications, has impaired renal function or prefers to avoid intravenous therapy. The main objective of outpatient management is to offer the right treatment to those most likely to benefit while limiting adverse effects, unnecessary prescribing and inequitable access [171,169].

Antiviral therapies

Nirmatrelvir inhibits the SARS-CoV-2 main protease, which is required for cleavage of viral polyproteins, whereas coadministered ritonavir enhances exposure through inhibition of drug metabolism. In the Evaluation of Protease Inhibition for COVID-19 in High-Risk Patients (EPIC-HR) trial conducted among unvaccinated, nonhospitalized high-risk adults, a five-day course significantly reduced COVID-19-related hospitalization or death when initiated early after symptom onset. This finding established proof of clinical efficacy in a susceptible population, but its magnitude cannot be directly generalized to highly vaccinated populations. In a subsequent randomized trial including vaccinated and standard-risk participants, the primary symptom-based endpoint did not show a significant benefit; interpretation depends on underlying risk, the rarity of severe outcomes, and eligibility characteristics. Recent guidance therefore supports risk-based selection rather than indiscriminate outpatient use [10,169,172].

Remdesivir is a nucleotide analogue prodrug that inhibits the viral RNA-dependent RNA polymerase. In the PINETREE placebo-controlled outpatient trial, a three-day intravenous course initiated within seven days of symptom onset lowered hospitalization or death among high-risk patients. Remdesivir is useful when clinically indicated oral therapies are unsuitable because of major drug interactions or other restrictions, although infusion access, transportation, and monitoring requirements can limit feasibility. The ACTT-1 trial demonstrated faster clinical recovery among adults hospitalized with COVID-19, most notably those requiring lower levels of respiratory support; conclusions regarding mortality benefit were less certain. Benefit in hospitalized patients should be interpreted in relation to disease stage, oxygen requirements, and whether persisting viral replication plausibly contributes to progression [173,174].

Molnupiravir promotes viral mutagenesis through incorporation of a nucleoside analogue into viral RNA. The MOVe-OUT trial in unvaccinated high-risk outpatients reported a modest reduction in hospitalization or death, but the larger PANORAMIC study, conducted predominantly among vaccinated adults, did not demonstrate a reduction in its primary hospitalization-or-death outcome despite faster self-reported recovery. Consequently, molnupiravir is generally considered a more limited option when preferred antivirals are unsuitable, and its use requires attention to reproductive safety restrictions and local product information. Other orally administered protease inhibitors, including simnotrelvir, have demonstrated virological or symptom-related effects in specific randomized populations, but

regional regulatory status and demonstration of benefit on severe clinical outcomes must be evaluated separately [175–177].

Across agents, treatment must be initiated while antiviral suppression can plausibly alter disease progression. A favorable effect on viral RNA trajectories should not be equated with a proven reduction in hospitalization, mortality, or long-term impairment. Comparative effectiveness studies across vaccinated populations, immunocompromised subgroups and contemporary lineages remain important because head-to-head randomized evidence is limited. Clinical pathways should incorporate the risk of treatment delay from prescription verification, renal function assessment, pharmacy access, and infusion availability. Where access is constrained, prioritizing vulnerable patients for timely therapy is more defensible than universal prescribing based solely on diagnosis [169,171].

Anti-inflammatory and immunomodulatory therapies

The beneficial role of systemic corticosteroids in hypoxemic COVID-19 is supported principally by the RECOVERY platform trial. Dexamethasone at 6 mg daily for up to ten days was associated with lower 28-day mortality among patients receiving invasive mechanical ventilation or supplemental oxygen, whereas no survival benefit was observed among participants without a respiratory-support requirement. These subgroup findings have important biological plausibility: attenuation of host-mediated injury becomes valuable during the inflammatory phase, whereas premature immunosuppression may be unnecessary or harmful during uncomplicated viral replication. Consequently, oxygen requirement and the trajectory of respiratory illness, rather than an isolated inflammatory laboratory result, remain central to corticosteroid selection. Hyperglycemia, delirium, secondary infections, and other corticosteroid-associated complications should be anticipated and monitored [11,170].

Targeted inhibition of interleukin-6 signaling improves outcomes in appropriately selected hospitalized patients with extensive inflammatory disease. In RECOVERY, addition of tocilizumab to standard care reduced 28-day mortality and the risk of invasive ventilation or death, with most participants receiving systemic corticosteroids. REMAP-CAP separately demonstrated improved organ support-free outcomes and survival with tocilizumab or sarilumab among critically ill patients treated soon after initiation of intensive-care organ support. These results support targeted immunomodulation in patients with severe or rapidly progressing disease rather than routine administration in mild infection. Contraindications, hematological abnormalities, hepatic dysfunction and possible bacterial or fungal infection require individualized risk-benefit evaluation [178,179].

Janus kinase inhibition provides a different mechanism for modulating cytokine signaling. RECOVERY found lower mortality with baricitinib in hospitalized COVID-19, while ACTT-2 reported shorter recovery time when baricitinib was combined with remdesivir in a trial conducted before corticosteroids became routine treatment. The 2026 IDSA guideline specifically compares baricitinib and tocilizumab when a clinician has determined that an additional immunomodulatory agent is indicated; the choice is informed by certainty of evidence, availability, contraindications and patient characteristics rather than assumptions of universal equivalence. These drugs should not be reflexively combined with multiple other immunomodulators without evidence for the specific regimen. Renal dosing, thrombosis risk, infectious complications and monitoring requirements warrant attention [180–182].

Treatment escalation should reflect a recognizable inflammatory phenotype and the speed of clinical deterioration. The optimal choice between different agents may be influenced by available trials, prior exposure, concurrent infections, organ dysfunction and local formularies. The evidentiary hierarchy also varies across indications: robust mortality trials underpin dexamethasone and selected immunomodulators, whereas many other proposed anti-inflammatory therapies have relied upon small, unblinded or biologically selected studies. Scientific reporting should avoid interpreting biomarker reduction alone as evidence of clinical efficacy. Continued research is needed on distinct immunopathological endotypes and on whether biomarker-guided treatment selection improves outcomes beyond clinical assessment and conventional severity classification [11,182].

Respiratory support and oxygen therapy

Hypoxemic respiratory failure is the principal indication for escalation of supportive treatment in severe COVID-19. Initial management requires evaluation of oxygen saturation, respiratory effort, gas exchange, hemodynamic stability and the possibility of alternative or concurrent causes of respiratory compromise. Conventional oxygen can be sufficient for mild hypoxemia; patients with greater oxygen needs may require high-flow nasal oxygen or continuous positive airway pressure (CPAP) in settings capable of close observation. In RECOVERY-RS, CPAP was associated with a reduction in the composite of tracheal intubation or mortality compared with conventional oxygen, whereas the corresponding result for high-flow nasal oxygen was less conclusive. Selection should remain individualized, recognizing that sustained respiratory effort despite apparently acceptable oxygen saturation may foreshadow deterioration [183].

Prone positioning can improve ventilation-perfusion matching and oxygenation, but its effects depend on patient selection and the clinical setting. A multinational randomized meta-trial among nonintubated adults receiving high-flow nasal oxygen found fewer instances of intubation or death with awake prone positioning, largely driven by a lower intubation rate, but did not establish a standalone mortality reduction. Awake proning requires patient cooperation, tolerance and adequate clinical supervision; it must not postpone intubation when the patient is deteriorating. Patients receiving invasive ventilation should be managed according to established acute respiratory distress syndrome principles, including lung-protective ventilation, appropriate positive end-expiratory pressure and prone positioning where indicated. Extracorporeal membrane oxygenation may be considered for carefully selected cases of refractory respiratory failure in experienced centers [183,184].

The principal clinical danger during noninvasive support is delayed recognition of treatment failure. A rising respiratory rate, sustained high work of breathing, altered consciousness, hemodynamic compromise or worsening gas exchange should prompt urgent reassessment. Excessive oxygen exposure, device-related pressure injury, aerosol-generating procedures and staffing constraints also deserve attention. Beyond respiratory rescue, early mobilization when feasible, nutritional support and prevention of intensive-care-acquired weakness form part of comprehensive care. High-resource respiratory technologies should not be presented as substitutes for timely escalation decisions, trained staff and reliable monitoring systems, particularly where critical-care capacity is limited [170,183].

Management of severe and critical COVID-19

Critical COVID-19 constitutes a heterogeneous syndrome characterized by severe hypoxemic respiratory failure, shock, acute kidney injury, thromboembolic events and occasionally dysfunction of multiple organ systems. Clinical management requires parallel attention to the viral infection, host inflammatory injury and the immediate physiological consequences of organ failure. Repeated assessment should include respiratory support requirements, fluid balance, hemodynamics, renal and hepatic function, neurological status, glycaemic control and evidence of secondary complications. For patients requiring supplemental oxygen, corticosteroids form the core of anti-inflammatory management; targeted agents may be added in selected rapidly progressing cases. The role of remdesivir is influenced by timing and severity, with uncertainty greatest among patients already requiring prolonged invasive ventilation [11,174,178,180].

Sepsis and shock management should follow established critical-care principles, including judicious resuscitation, assessment of fluid responsiveness and vasopressors when indicated. The presence of systemic inflammation alone is not evidence of bacterial sepsis. When a patient develops new instability, clinicians should actively investigate alternative etiologies, such as pulmonary embolism, bacterial superinfection, myocardial dysfunction or medication-related toxicity. Renal replacement therapy, sedation practices and ventilator liberation should be guided by general critical-care evidence rather than presumptions that all organ dysfunction is unique to COVID-19. Protocols must also preserve opportunities for communicating goals of care and shared decisions with patients and families [170,185].

Treatment success should be evaluated by survival, functional recovery and the avoidance of organ support, not exclusively by improvements in oxygenation or inflammatory biomarkers. Trial evidence from early variants, during periods with limited prior immunity, remains informative but requires interpretation in light of contemporary case mix. Many patients now

admitted with a positive SARS-CoV-2 test have complex comorbidities or concurrent diagnoses that alter the relative contribution of acute viral pneumonia. Avoiding both undertreatment of genuine COVID-19-related organ failure and overtreatment of incidental infection is therefore a central contemporary challenge [169,182].

Prevention and management of thromboembolic complications

COVID-19-associated inflammation, endothelial activation, immobility and critical illness increase the risk of venous thromboembolism, but the intensity of anticoagulation should be supported by evidence rather than D-dimer elevation alone. Standard pharmacological thromboprophylaxis is indicated for many hospitalized patients without prohibitive bleeding risk, consistent with general inpatient thrombosis-prevention principles. In the INSPIRATION randomized trial among critically ill patients, intermediate-dose prophylaxis did not reduce the composite of thrombosis, extracorporeal membrane oxygenation or mortality compared with standard prophylaxis, while raising safety concerns. Routine empirical dose escalation for all intensive-care patients is therefore unsupported [170,186].

Randomized multiplatform investigations demonstrated important differences between clinical severity strata. Therapeutic-dose heparin improved the probability of survival to discharge without organ support among selected noncritically ill hospitalized patients, although the benefit must be balanced against increased bleeding. In contrast, among critically ill patients, therapeutic-dose anticoagulation did not confer the anticipated organ-support or survival advantage relative to usual thromboprophylaxis and resulted in more major bleeding events. These findings should not be reduced to a general recommendation for full-dose anticoagulation in every hospitalized patient; eligibility, bleeding risk, local protocols and concurrent clinical indications remain decisive [187,188].

When venous thromboembolism is suspected, investigation should be determined by symptoms, hemodynamic status, imaging availability and clinical probability. D-dimer has poor specificity in patients with active inflammation, and elevated concentrations alone should not trigger therapeutic anticoagulation. Patients with confirmed thrombosis require treatment according to conventional therapeutic indications, with attention to renal function, platelet count, interactions and procedural needs. Decisions concerning post-discharge prophylaxis should account for persistent immobility, previous thrombosis and bleeding risk; routine use after discharge is not justified for all patients with prior COVID-19 hospitalization. Research priorities include identifying subsets with persistent prothrombotic risk despite widespread population immunity [186–188].

Management of secondary infections and antimicrobial stewardship

Bacterial coinfection at presentation and secondary infection during hospitalization are distinct entities that require different diagnostic approaches. An early living meta-analysis demonstrated a relatively low prevalence of bacterial coinfection despite frequent prescribing of antibiotics, indicating substantial potential for antimicrobial overuse. The probability of secondary infection becomes more relevant with prolonged hospitalization, invasive ventilation, immunosuppression and new clinical deterioration. Fever, leukocytosis and C-reactive protein elevation are not specific evidence of bacterial disease because they may also reflect COVID-19-associated inflammation. Accordingly, routine antibacterial treatment is inappropriate for uncomplicated viral infection without clinical suspicion of bacterial coinfection [170,185].

Where bacterial superinfection is suspected, cultures should be obtained when appropriate and before antimicrobial administration if doing so does not create a clinically important delay. Empirical therapy should be guided by the likely source, patient acuity, prior antimicrobial exposure, local resistance patterns and risk factors for multidrug-resistant pathogens. Treatment should be reassessed when culture results, imaging and the clinical course become available, with narrowing or discontinuation when bacterial infection is unsupported. Immunomodulatory treatment creates additional diagnostic complexity because fever and inflammatory biomarkers may be suppressed. Clinicians should also recognize the risks of opportunistic fungal infections in selected critically ill or immunosuppressed patients without assuming that fungal prophylaxis benefits all individuals receiving COVID-19 therapies [182,185].

The pandemic demonstrated how diagnostic uncertainty, limited microbiological capacity and fear of clinical deterioration can promote unnecessary antibiotics. These prescribing patterns have particular implications for antimicrobial resistance in low- and middle-income countries, where access to rapid diagnostics, microbiology and antimicrobial stewardship teams may be constrained. Integrating diagnostic sampling, local antibiograms, review of antibiotic duration and routine reassessment into COVID-19 clinical pathways can reduce avoidable exposure without compromising timely management of genuine bacterial sepsis. Antibiotic prescribing quality should be considered a core measure of COVID-19 healthcare delivery, rather than an optional component of supportive care [170,185].

Clinical management in special populations

Patients with impaired humoral immunity, hematological malignancies, organ transplants or B-cell-depleting treatment may remain susceptible to severe and persistent SARS-CoV-2 infection despite previous vaccination. Antiviral treatment should be considered promptly in individuals at substantial risk; however, immunosuppressive medications, organ dysfunction and potential drug interactions may restrict oral treatment choices. Persistent infection should not be confused with post-infectious symptoms or a new infection solely on the basis of repeated positive polymerase chain reaction results. Serial clinical assessment and, when available, virological sequencing may clarify whether ongoing replication is occurring. A 2026 individual-patient-data review reported viral clearance after extended nirmatrelvir/ritonavir-containing regimens in selected persistently infected patients, but its small observational evidence base does not establish comparative effectiveness or a universal extended regimen [189].

A 2025 case series described clinical and virological responses to prolonged or combined antiviral therapy among B-cell-depleted patients with protracted infection. These results provide a rationale for specialist-directed individualized management but do not supersede randomized evidence or justify routine combination therapy for all immunocompromised patients. The clinical problem is particularly complex when viral evolution occurs within an individual and when there is risk of resistance under prolonged antiviral exposure. Immunocompromised patients may also require multidisciplinary collaboration among infectious disease, hematology, oncology and transplant specialists to balance antiviral efficacy against transplant or cancer-treatment considerations [189–190].

Children and adolescents have different baseline risks and treatment eligibility thresholds from adults. A Pediatric Infectious Diseases Society consensus statement emphasizes identification of pediatric patients at high risk, age- and weight-appropriate prescribing and avoidance of extrapolating every adult treatment indication to otherwise healthy children. For pregnant patients, maternal oxygenation and timely management of severe disease remain essential, while available evidence and safety considerations should be reviewed for each agent. Treatment should not be withheld reflexively in a high-risk pregnant patient solely because randomized pregnancy-specific trials are limited, but individual medication labeling and obstetric consultation remain important. Older adults, persons with frailty and patients with substantial renal or hepatic impairment require similar attention to net clinical benefit and polypharmacy [169,170,191].

Treatment limitations, adverse effects, drug interactions and antiviral resistance

Although antiviral agents offer clinically meaningful benefits for selected patients, prescribing is complicated by drug interactions, contraindications and the evolving natural history of infection. Ritonavir is a potent inhibitor of cytochrome P450 3A and can substantially alter exposure to commonly prescribed medicines, including selected antiarrhythmics, anticoagulants, anticonvulsants, sedative agents and immunosuppressants. A structured interaction review is essential before nirmatrelvir/ritonavir administration, with temporary interruption, dose modification, enhanced monitoring or selection of an alternative antiviral when clinically appropriate. Not every interacting medicine can be safely paused. Renal function and established local prescribing information should be reviewed because nirmatrelvir dosing and contraindications depend upon kidney function [169,192].

Clinical or virological rebound after an apparent initial response has been reported in treated and untreated individuals. In a prospective observational study, culture-based or viral-load-defined rebound was observed among a subset of nirmatrelvir/ritonavir recipients and could occur without recurrent symptoms. Such findings do not demonstrate that treatment routinely fails, nor do they justify extending antiviral therapy for all patients. Rebound definitions vary widely across studies, contributing to inconsistent incidence estimates. Ongoing infectiousness should be assessed using clinical context and infection-control guidance; a single cycle-threshold value cannot independently characterize transmissibility. Studies of prolonged replication in immunocompromised hosts underscore the importance of distinguishing drug resistance, rebound and persistent infection [190,193].

Widespread antiviral deployment also creates selective pressure for variants with altered drug susceptibility. Laboratory demonstrations of reduced susceptibility must be interpreted with evidence on viral fitness, prevalence and treatment-associated clinical failure. Routine whole-genome sequencing is not required before prescribing standard early antivirals, but targeted testing can be valuable in prolonged infection, suspected resistance or unusual epidemiological clusters. The development of noninteracting antiviral regimens, novel protease and polymerase inhibitors and feasible treatment combinations may address important gaps. Future clinical trials should quantify adverse events and patient-important outcomes as carefully as virological responses, with prespecified analyses for immunocompromised hosts [190,192].

Emerging therapeutic strategies and investigational treatments

The evolving therapeutic pipeline includes novel inhibitors of SARS-CoV-2 replication, antibody-based products, host-directed interventions and strategies specifically designed for persistent infection. Ensitrelvir, an oral main protease inhibitor, was evaluated in the global SCORPIO-HR randomized trial. The prespecified primary symptom-resolution endpoint was not significantly improved, even though virological outcomes indicated antiviral activity; this distinction illustrates why reductions in viral load are not sufficient to establish a patient-important treatment benefit. Simnotrelvir has also been studied in randomized trials, but evidence about prevention of hospitalization or mortality in vaccinated high-risk populations remains less definitive than for agents established through severe-outcome trials. Geographic differences in authorization further limit any assumption of universal availability [177,194].

Antibody-based therapies require continuing reassessment because susceptibility of circulating variants changes with spike-protein evolution. Many monoclonal antibodies developed early in the pandemic lost neutralizing activity against later variants and should not be assumed active on the basis of historical clinical results. By contrast, high-titer convalescent plasma remains of interest in carefully selected individuals unable to mount an adequate antibody response. A 2025 randomized European study among clinically vulnerable, largely vaccinated immunocompromised patients found fewer hospitalizations or deaths after early very-high-titer plasma compared with standard care, although the sample was relatively small and findings should be interpreted in relation to product potency and the study population. Plasma therapy is not a substitute for validated antivirals in ordinary-risk cases [190,195].

The therapeutic record also demonstrates the value of de-implementation. Randomized trials did not support ivermectin for accelerating sustained recovery in outpatients, and the RECOVERY hydroxychloroquine trial failed to show a mortality benefit in hospitalized patients. Inadequate empirical use of ineffective agents can delay evidence-supported interventions and divert limited resources. Future studies should prioritize trials with clinically meaningful outcomes, variants and host-immunity strata relevant to contemporary care, transparent safety monitoring and sufficient representation of pregnant patients, children, immunocompromised individuals and lower-resource health systems. Adaptive multicenter trial platforms provide a means of responding to evolving variants while retaining methodological rigor [194,196,197].

Ultimately, contemporary COVID-19 management requires coordinated deployment of proven antivirals in early disease, immunomodulation in selected inflammatory illness, appropriate organ support and prevention of iatrogenic complications. Despite substantial treatment advances, important gaps persist in comparative antiviral effectiveness, duration of therapy for persistent infection, management of highly vulnerable populations and global access. A high-quality evidence base must continuously distinguish laboratory plausibility from verified

clinical effectiveness and should reflect changes in baseline risk created by vaccination, previous infection and viral evolution [169,182,189].

Post-COVID-19 condition (long COVID)

Definitions and diagnostic criteria of long COVID

Post-COVID-19 condition (PCC), commonly termed long COVID or post-acute sequelae of SARS-CoV-2 infection (PASC), encompasses new, persistent, or relapsing health problems occurring after acute SARS-CoV-2 infection. The World Health Organization consensus definition describes a condition in individuals with probable or confirmed infection, usually occurring three months after disease onset, with symptoms lasting at least two months and not attributable to an alternative diagnosis [12]. This definition provides an operational framework for research and clinical surveillance rather than a definitive biological boundary. Clinical syndromes beginning earlier, fluctuating after apparent recovery, or manifesting as objectively documented organ injury also warrant consideration, although they may not meet every research definition. The broader concept of long COVID consequently includes overlapping, but not interchangeable, entities: persistent symptoms, new postinfectious diagnoses, and sequelae of severe acute illness [13].

The lack of a single pathognomonic feature complicates case ascertainment. Prolonged fatigue, cognitive dysfunction, exertional intolerance, dysautonomia, and altered smell or taste are clinically informative but not individually specific to SARS-CoV-2 infection. Symptom-based definitions must account for the frequency of similar complaints in uninfected populations, pre-existing morbidity, and the physiological consequences of critical illness. In a prospective multicentre cohort, the updated 2024 RECOVER-adult research index incorporated post-exertional malaise, fatigue, brain fog, dizziness, palpitations, respiratory complaints, altered smell or taste, and other manifestations; it identified distinct symptom clusters associated with impaired health-related quality of life [198]. Importantly, this index was designed for research classification and should not be treated as a mandatory clinical diagnostic threshold.

Age-appropriate definitions are equally important. Children may present with school impairment, abdominal pain, headaches, sleep disturbances, reduced exercise tolerance, or neurocognitive symptoms that differ from adult phenotypes. The RECOVER pediatric cohort identified distinguishable patterns among school-age children and adolescents, supporting the use of developmental-stage-specific assessments instead of directly applying adult scoring systems [199]. Throughout the following discussion, long COVID is used as the overarching clinical term, while PCC refers more specifically to studies applying the WHO time-based criteria. Differences in case definition are treated as a principal explanation for between-study heterogeneity.

Epidemiology and global burden

The epidemiological burden of long COVID is substantial, but its precise magnitude remains uncertain. Estimates depend on the denominator used, the interval since infection, symptom attribution, study recruitment, variant period, vaccination status, and whether an uninfected comparator population was included. An updated systematic review encompassing 429 meta-analyzed studies reported a pooled prevalence of 36% (95%CI: 33%–40%) across heterogeneous definitions and follow-up periods [200]. A separate 2026 synthesis estimated a pooled prevalence of 30.8% (95%CI: 26.8%–35.0%), but reported extreme heterogeneity (I^2: 99.7%) and a wide prediction interval [201]. These estimates cannot be interpreted as stable probabilities that an individual infected in 2026 will develop WHO-defined PCC. They describe selected populations, frequently including earlier pandemic periods and broader symptom-based outcomes.

Variant era and pre-existing immunity are central epidemiological modifiers. In a large electronic health-record analysis, the post-acute burden differed across pre-Delta, Delta, and Omicron periods and was generally lower among vaccinated than unvaccinated individuals, although residual risk remained in the Omicron era [202]. Nevertheless, lower relative risk per infection does not necessarily imply negligible population burden when infections are frequent. Moreover, mild outpatient infections contribute to the absolute number of affected individuals because they are substantially more common than critical illness. Cross-national comparisons are

further distorted by differential access to polymerase chain reaction testing, follow-up services, electronic records, and compensation or disability systems.

Longitudinal observations indicate that long COVID is not invariably transient. A US Veterans Affairs analysis found diminishing but persisting incident post-acute disease burden over three years, particularly among people hospitalized during acute infection; interpretation requires recognition of the cohort's demographic profile and potential residual confounding [203]. Among patients discharged after hospitalization early in the pandemic, two-year follow-up identified enduring symptoms and reduced health status in subsets of survivors, emphasizing the need to differentiate post-intensive-care disability and organ injury from symptom-predominant PCC [204]. Estimation of contemporary burden should therefore incorporate representative sampling, infection-negative control groups, standardized follow-up intervals, and measurements of both symptom persistence and functional impairment.

Risk factors and vulnerable populations

A broad range of host and infection-related characteristics have been associated with long COVID, although effect sizes vary by design and phenotype. A 2025 systematic review of prospective cohorts identified female sex and hospitalization during acute infection as recurrent risk factors, while reporting substantial between-study variability [205]. Older age, obesity, chronic cardiometabolic disease, higher acute symptom burden, and socioeconomic disadvantage may also contribute, but associations are sensitive to adjustment strategies and the ascertainment of pre-existing symptoms. Some individuals who develop disabling long COVID were previously healthy and experienced only mild acute disease; therefore, risk stratification should not be used to exclude evaluation in low-risk groups.

Repeated infections create an additional dimension of susceptibility. A large observational analysis associated SARS-CoV-2 reinfection with additional acute and post-acute adverse outcomes, although causal interpretation is constrained by differences in healthcare utilization and the underlying vulnerability of repeatedly infected groups [206]. Vaccinated individuals can also develop long COVID after breakthrough infection. In a population-based analysis, vaccination was associated with lower post-acute risks than infection in unvaccinated individuals but did not eliminate sequelae [209]. Comparisons across vaccination strata must account for confounding by prior infection, vaccine access, treatment availability, and calendar period.

The natural history of individual phenotypes also influences apparent risk. In a prospective cohort with two-year observation, trajectories varied across patients who met the WHO definition, with some showing persistent symptoms or fluctuating recovery [208]. In clinical practice, vulnerable populations include older adults with limited physiological reserve, individuals with pre-existing cardiopulmonary or neurological disorders, people with immunodeficiency, pregnant individuals, children, and those facing barriers to access. Equitable identification requires attention to underdiagnosis in low-resource settings, where the absence of documented SARS-CoV-2 testing may preclude formal recognition despite a compelling infection history.

Proposed pathophysiological mechanisms

Long COVID is increasingly considered a group of intersecting postinfectious disorders rather than a single pathogenetic entity. Proposed mechanisms include persistence of viral material in anatomical reservoirs, prolonged innate and adaptive immune activation, altered microbial communities, endothelial injury, dysautonomia, metabolic dysfunction, and secondary effects of tissue damage. A synthesis of mechanistic and clinical evidence emphasized that these pathways may coexist and differ according to symptom phenotype, time since infection, and baseline susceptibility [209]. None has yet been validated as a universal mechanism or diagnostic marker, and associations identified in small mechanistic cohorts should not be equated with proof of causality.

Tissue-based investigations provide biologically plausible evidence for persistent antigenic stimulation. In a cross-sectional cohort following mild infection, SARS-CoV-2 RNA was detectable in selected tissue specimens months after apparent clinical recovery and was associated with persistent symptoms, although tissue positivity does not demonstrate replication-competent virus or establish causation [210]. Molecular imaging and tissue sampling have also

revealed prolonged immune-cell activation and viral RNA in some participants long after acute infection; importantly, elevated immune activation was observed in certain individuals without long COVID symptoms [211]. These findings support further study of tissue reservoirs but do not justify routine invasive testing or prolonged antiviral therapy outside appropriate research settings.

Immune profiling has identified alterations in myeloid and lymphocyte populations, inflammatory mediators, antiviral antibody responses, and endocrine measures among people with long COVID [212]. Proteomic analyses have additionally implicated complement dysregulation and thromboinflammatory signaling, suggesting interactions between immune activation and the vascular system [213]. Although these findings offer candidate biological pathways, case-control selection, sampling time, concurrent medications, and biological variability limit immediate translation into diagnostic assays. In particular, microvascular hypotheses should not be taken as evidence that empiric therapeutic anticoagulation is effective or safe for symptom-predominant long COVID.

Metabolic and neuromuscular abnormalities may contribute to exercise intolerance. A longitudinal study of participants with post-exertional malaise reported skeletal-muscle structural and metabolic changes that worsened after exertion, providing a physiological explanation for symptom exacerbation in a subgroup [214]. Neuroimmune and gut-brain mechanisms have also been proposed. A translational investigation linked peripheral serotonin changes to viral-infection-associated inflammation and cognitive dysfunction [215]; however, a subsequent independent clinical study did not replicate lower serum serotonin concentrations in a smaller long COVID cohort [216]. This divergence illustrates why promising candidate pathways require external validation. Contemporary models integrate potential viral persistence, inflammatory signaling, metabolic disruption, and multisystem dysfunction without assuming that any one mechanism explains all cases [217].

Clinical phenotypes and multisystem manifestations

The clinical presentation is heterogeneous, variable over time, and frequently multisystemic. Fatigue may coexist with non-restorative sleep, impaired concentration, headaches, myalgia, arthralgia, altered smell or taste, chest discomfort, dyspnoea, palpitations, gastrointestinal disturbances, and psychological distress. Post-exertional symptom exacerbation is particularly consequential: patients may experience a delayed and disproportionate deterioration after physical, cognitive, or emotional effort. Its recognition distinguishes a subgroup for whom conventional exercise escalation may be poorly tolerated. Severity should be assessed by interference with normal functioning, not exclusively by the number of reported symptoms [13,198].

Cardiopulmonary manifestations require separation of structural organ disease from functional limitation. Persistent dyspnoea may reflect residual parenchymal abnormalities, impaired gas transfer, deconditioning, dysfunctional breathing, or autonomic mechanisms; several processes may operate simultaneously. Cardiovascular complaints include tachycardia, orthostatic intolerance, and exertional symptoms, whereas other individuals have clinically demonstrable cardiac or thromboembolic complications. Autonomic dysfunction, including postural orthostatic tachycardia syndrome, has been proposed as an important component of some long COVID phenotypes, but prevalence estimates vary because formal autonomic testing is not consistently performed [218].

Neurocognitive manifestations include slowed information processing, impaired working memory, attentional difficulties, headache, dysosmia, sleep disturbance, and sensory symptoms. Their assessment requires consideration of depression, anxiety, insomnia, medication effects, nutritional deficiencies, and pre-existing neurological conditions without assuming that these explain all postinfectious symptoms. Children and adolescents may exhibit reduced school attendance, developmental or behavioral changes, abdominal symptoms, and fatigue; data from Indonesia have also described pediatric PASC presentations, although available cohorts are too limited to quantify national burden reliably [219]. Clinical phenotyping should consequently document symptom onset, trajectories, functional impact, objective abnormalities, and the possibility of coexisting diagnoses.

Diagnostic approaches and clinical assessment

There is currently no validated single laboratory test, imaging feature, or clinical biomarker that establishes long COVID across its heterogeneous phenotypes. Diagnosis remains clinical and depends on a credible temporal relationship with SARS-CoV-2 infection, persistence or emergence of compatible manifestations, functional effects, and a proportionate assessment for alternative explanations. A previously documented positive virological test is helpful but should not be an absolute requirement when historical access to testing was limited. Contemporary clinical guidance supports comprehensive history-taking, physical examination, targeted laboratory investigations, and phenotype-specific functional assessment rather than indiscriminate testing [220].

Initial evaluation should establish the course of acute illness, vaccination and reinfection history, pre-infection functional status, medication exposure, symptom onset and fluctuation, and key warning signs. Basic investigations may be guided by the presentation, such as complete blood count, renal and hepatic chemistry, thyroid function, markers of anemia or nutritional deficiency, and selected inflammatory parameters. Persistent respiratory complaints may warrant pulmonary function testing, oxygen saturation assessment, or chest imaging; orthostatic symptoms may justify bedside standing measurements or referral for autonomic evaluation. Cardiac imaging, rhythm monitoring, neuropsychological assessment, and sleep investigations should be reserved for compatible indications. Routine panels of unvalidated cytokines, autoantibodies, or viral persistence assays are not supported for general diagnostic use.

The differential diagnosis is clinically substantial. Residual lung injury, pulmonary embolism, myocarditis, endocrine disease, anemia, medication adverse effects, primary sleep disorders, new autoimmune or neurological disease, and complications of critical illness can mimic or coexist with long COVID. When red-flag features such as progressive focal neurological deficits, syncope, resting hypoxemia, severe chest pain, or rapidly deteriorating exercise tolerance are present, urgent evaluation of conventional acute disease pathways takes precedence over attribution to a postinfectious syndrome. Symptom and quality-of-life instruments are valuable for follow-up but should not replace individual clinical judgment.

Impact on quality of life, functional status, and socioeconomic outcomes

The burden of long COVID extends beyond organ-specific morbidity. Persistent fatigue, cognitive limitations, exertional intolerance, and pain can restrict employment, education, caregiving, and participation in social life, while repeated investigations and uncertain prognosis may add psychological and financial strain. Functional disability may be pronounced even when conventional imaging or routine laboratory tests are normal. These consequences are especially relevant for working-age adults and for people with limited paid leave, access to rehabilitation, or social protection.

Economic analyses suggest measurable productivity losses associated with the condition, but such estimates are context-specific and highly dependent on case definition and national labor markets. A 2025 US analysis estimated substantial additional lost labor costs among full-time workers reporting long COVID, reinforcing that indirect costs may be considerable even without hospitalization [221]. These findings should not be directly extrapolated to low- and middle-income countries, where informal employment, unpaid domestic work, and financial vulnerability may alter the magnitude and distribution of losses.

Recovery trajectories reinforce the need for longitudinal functional outcomes rather than cross-sectional symptom prevalence alone. In a 2026 prospective analysis of 3,590 individuals attending a specialist post-COVID service, only a subset attained satisfactory self-reported recovery over four years, highlighting substantial ongoing service needs; referral selection limits generalization to all people infected with SARS-CoV-2 [222]. Future burden assessments should jointly measure health-related quality of life, productivity, household costs, school participation, healthcare utilization, and equity in access to care.

Current management and rehabilitation strategies

Management is currently centered on individualized, symptom-directed and function-oriented care, preferably through coordinated primary care and multidisciplinary referral when needed. No intervention has demonstrated consistent disease-modifying efficacy across all long COVID

phenotypes. Clinical priorities include exclusion and treatment of alternative or coexisting disease, shared goal setting, rehabilitation adapted to symptoms, management of sleep disturbance and pain, appropriate psychological support, and evaluation of orthostatic or respiratory problems. Education should acknowledge uncertainty without implying that symptoms are necessarily psychogenic or that normal routine investigations exclude physiological dysfunction [220].

Rehabilitation evidence is evolving and should be interpreted by population and symptom phenotype. In the REGAIN multicentre randomized trial, an online supervised program combining physical and psychological rehabilitation improved selected health-related quality-of-life outcomes among adults following COVID-19 hospitalization [223]. However, intervention benefits from a post-hospital cohort should not be generalized uncritically to patients with prominent post-exertional malaise. Exercise prescriptions require screening for symptom exacerbation, individualized pacing, and adjustment based on recovery; automatic escalation of exercise intensity is inappropriate in patients whose symptoms reliably worsen after exertion. Respiratory physiotherapy, occupational therapy, cognitive rehabilitation, and activity planning may be helpful when linked to specific impairments.

Evidence for pharmacological disease modification remains limited. In the placebo-controlled STOP-PASC trial, a 15-day course of nirmatrelvir/ritonavir did not significantly improve a composite of long COVID symptoms in a predominantly vaccinated cohort with prolonged symptom duration [224]. This negative result does not exclude a potential effect in other, biologically selected subgroups, but argues against routine prescribing solely for established long COVID. Pharmacological treatment of tachycardia, neuropathic pain, sleep disorders, or other manifestations may be considered on conventional clinical indications and after assessment of adverse effects. A scoping review of autonomic dysfunction treatments found predominantly small, uncontrolled studies and insufficient evidence for broad claims of efficacy [225].

Prevention of long COVID and the role of vaccination

Prevention principally involves reducing the likelihood of SARS-CoV-2 infection and clinically consequential reinfection, protecting high-risk populations, and providing evidence-based treatment during acute illness. The risk of long COVID is not eliminated by a mild initial illness; prevention strategies should therefore consider cumulative infection burden alongside mortality and hospitalization outcomes. Risk reduction attributable to acute antiviral treatment remains an area of active investigation, and observational associations should not be assumed to establish efficacy against long COVID itself.

One randomized trial offers evidence that an acute-phase intervention may influence later post-COVID outcomes. In the COVID-OUT trial, early outpatient metformin among adults with overweight or obesity was associated with a lower cumulative incidence of a clinician-diagnosed long COVID outcome over ten months (hazard ratio: 0.59; 95%CI: 0.39–0.89) [226]. Interpretation should account for the trial's selected population, timing of treatment, and the use of clinician-diagnosed long COVID as a secondary outcome; the finding is not sufficient to recommend metformin universally for prevention irrespective of eligibility, contraindications, or current guidance.

Vaccination is associated with a lower probability of long COVID in several observational datasets, although the strength of evidence depends on dose, variant, exposure history, and study design. A systematic review and meta-analysis of observational studies reported lower odds following a two-dose pre-infection vaccine regimen (summary odds ratio (OR): 0.76; 95%CI: 0.65–0.89), while uncertainty remained for several other dose and post-infection comparisons [227]. Vaccination should therefore be discussed as part of an integrated risk-based prevention strategy rather than as a guarantee against long COVID. Updated vaccination programs may confer indirect benefits through prevention of severe acute disease, but the long-term effectiveness of variant-updated formulations against chronic sequelae requires dedicated evaluation.

Unresolved questions and research priorities

Several interrelated limitations constrain clinical translation. Definitions vary substantially, infection histories are incompletely documented, comparison groups are often absent, and cohorts assembled in specialist services are vulnerable to referral and selection bias. Many studies were conducted before widespread hybrid immunity or during early variant periods, limiting their direct applicability to contemporary infections. Rigorous longitudinal studies should include well-characterized infected and uninfected controls, standardized phenotype definitions, biological sampling before and after infection where feasible, and prespecified endpoints that distinguish symptoms, objective organ injury, and functional impairment [200–202].

Mechanistic priorities include determining whether viral material represents biologically active reservoirs, establishing temporal directionality between persistent immune activation and symptoms, and identifying reproducible biomarkers that discriminate clinically meaningful phenotypes. Tissue studies, multiomics and longitudinal immune phenotyping could inform targeted treatment trials, but discovery cohorts must be externally validated across age, sex, geographic region, vaccination history, and variant period. Mechanism-specific randomized trials should test interventions with appropriate patient selection, objective and patient-reported outcomes, sufficient follow-up, and adequate monitoring for treatment-related harms [209–217].

Healthcare delivery research is also essential. Long COVID services should be assessed for effectiveness, cost, scalability, and equitable access, especially where primary care and rehabilitation capacity are limited. Future investigations must address children, pregnancy-associated presentations, older adults, immunocompromised individuals, and low- and middle-income settings, with outcomes extending beyond symptom counts to education, livelihood, quality of life, and participation. Ultimately, progress will require a shift from broad symptom labels toward reproducible clinical phenotypes linked to mechanisms and validated interventions, while maintaining timely supportive care for patients in whom the biology remains incompletely understood [12,13,222].

Prevention and control of COVID-19

Principles of COVID-19 prevention and control

The prevention of COVID-19 has evolved from emergency containment of a newly recognized respiratory pathogen to sustained, risk-adapted protection against a continually evolving virus. The principal objectives of contemporary prevention are to avert deaths and severe illness, preserve healthcare capacity, limit transmission in settings involving vulnerable individuals, and reduce the cumulative consequences of infection and reinfection. The relative contribution of each intervention depends on prevailing viral characteristics, existing population immunity, environmental conditions, healthcare access, and the distribution of clinical risk. Accordingly, a uniform intervention applied indiscriminately to all populations is unlikely to provide an optimal balance of effectiveness, acceptability, cost, and proportionality [228].

A layered prevention framework integrates vaccination, adequate ventilation and indoor air quality, appropriate respiratory protection, timely testing, measures to reduce exposure during infectious periods, and infection prevention and control in healthcare facilities. These interventions are complementary rather than mutually exclusive: vaccination substantially modifies the probability of severe clinical outcomes but does not establish sterilizing immunity, whereas interventions that reduce inhaled infectious aerosol concentrations can lower exposure independently of immune status. Effectiveness assessments should distinguish individual protection from population-level impact and separate mechanistic plausibility from results demonstrated in well-designed field studies [77,228,229].

Risk stratification remains central to equitable policy. Older adults, individuals with immunocompromising conditions, residents of long-term care facilities, and patients with major cardiopulmonary or metabolic disease have greater absolute risks of hospitalization or death, although risk varies substantially within each category. Prevention priorities should also consider pregnant individuals, children with complex underlying disease, healthcare workers, and persons facing structurally determined barriers to timely care. Measures designed for high-transmission institutional environments are not necessarily required in every community setting; instead,

policy should respond to local epidemiology, capacity constraints, and the potential harms of highly disruptive restrictions [13,24,228].

Non-pharmaceutical preventive interventions

Non-pharmaceutical interventions (NPIs) remain relevant because SARS-CoV-2 is transmitted predominantly through inhalation of virus-containing respiratory particles, particularly in crowded or poorly ventilated indoor environments. Well-fitting respirators with high filtration efficiency can reduce the concentration of particles inhaled by susceptible persons, whereas masks worn by infectious individuals provide source control. A rapid systematic review found that most included observational investigations associated masking or mask requirements with lower transmission; however, many studies had serious risk of bias and were conducted before Omicron predominance [229]. A subsequent synthesis of real-world masking policies likewise reported heterogeneous findings, illustrating the importance of adherence, respirator fit, background incidence, and concurrent interventions rather than treating mask use as a binary exposure [230].

Ventilation is an upstream exposure-control strategy that does not rely on identifying every infectious individual. Outdoor air supply, appropriately maintained mechanical ventilation, portable high-efficiency particulate air filtration, and reduction of crowding can mitigate accumulation of airborne particles. Environmental modeling and field investigations support these measures, but estimates of their independent clinical effectiveness remain heterogeneous because air exchange, room occupancy, filtration performance, and exposure duration are often measured inconsistently [77,231]. Carbon dioxide concentration may indicate the adequacy of ventilation relative to occupancy, but it does not directly measure viable SARS-CoV-2 concentration and must not be interpreted as a standalone infection-risk metric. Engineering measures should be adapted to local building conditions and sustained maintenance capacity.

Hand hygiene, respiratory etiquette, cleaning of frequently touched surfaces, and staying away from high-risk settings while acutely symptomatic remain reasonable components of integrated infection control. Their relative importance should reflect the predominance of airborne transmission: environmental disinfection alone cannot substitute for ventilation or respiratory protection. Healthcare-associated prevention additionally requires appropriate respirators during relevant clinical exposures, eye protection when indicated, attention to aerosol-generating procedures, staff training, and institutional protocols for isolation and patient placement. Systematic evaluations of personal protective equipment are informative but commonly combine several measures, making the causal effect of individual components difficult to isolate [232].

Testing and temporary exposure reduction can be targeted to symptomatic illness, outbreaks, vulnerable households, or anticipated contact with highly susceptible individuals. A negative antigen test obtained early after exposure cannot reliably exclude developing infection, and repeated testing may be justified when decisions have substantial clinical consequences [145–153]. Large-scale closure policies and prolonged restrictions may reduce contact opportunities but impose educational, social, and economic costs; their application should therefore be justified by clearly defined threats and proportional benefits rather than routinely reintroduced as default measures.

COVID-19 vaccination

Vaccination is the central biomedical intervention for preventing severe COVID-19. Its scientific basis is the induction of antigen-specific humoral and cellular responses capable of restricting viral replication and moderating inflammatory tissue injury after exposure. The clinical objective has shifted progressively from preventing symptomatic infection in immunologically naive populations to maintaining protection against hospitalization, critical illness, and death among populations with variable combinations of vaccination- and infection-derived immunity. Historical estimates from the original randomized trials cannot be assumed to represent contemporary effectiveness against antigenically divergent Omicron descendants [8,9,19,101,115]. The major COVID-19 vaccine platforms, immune responses, effectiveness considerations, and safety limitations are summarized in **Table 6**.

Table 6. COVID-19 vaccines: platforms, immune responses, effectiveness, and safety

Platform/strategy (examples)	Mechanism and principal immune responses	Effectiveness and implementation considerations	Safety and unresolved limitations	References
First-generation mRNA (BNT162b2; mRNA-1273)	Lipid nanoparticles deliver spike-encoding mRNA; induce binding/neutralizing antibodies and spike-specific T cells.	High efficacy against symptomatic ancestral-strain COVID-19 in pre-Omicron randomized trials; subsequent effectiveness depends on immune history and antigenic match.	Common transient reactogenicity; rare myocarditis/pericarditis with age-, sex-, and product-specific risk patterns.	[8, 101, 233, 243]
Variant-updated mRNA vaccines (XBB.1.5-, JN.1-/KP.2-related formulations)	Updated spike antigen boosts memory B-cell responses and neutralization against more recent variants.	Observational evidence supports additional protection against hospitalization and severe in-hospital outcomes, but estimates vary by lineage, time since vaccination and risk group.	Waning protection against infection and antigenic turnover necessitate ongoing formulation and effectiveness evaluation.	[236–240, 263]
Recombinant spike/protein subunit (NVX-CoV2373 and related)	Purified spike antigen plus adjuvant elicits humoral immunity and T-cell responses.	Efficacy established for early formulations; revised antigens and manufacturing schedules must be evaluated against current variants.	Reactogenicity and rare adverse events require continued monitoring; distribution and formulation availability differ by country.	[234, 235]
Nonreplicating viral-vector vaccines	Adenoviral vector delivers the spike gene, triggering humoral and cellular responses.	Important for early global vaccine access; performance against infection differs by variant and schedule.	Rare vaccine-induced thrombotic thrombocytopenia associated with some adenoviral products; use depends on current country policy.	[235, 244]
Inactivated whole-virus vaccines	Chemically inactivated virus presents multiple antigens, commonly with an adjuvant.	Broadly used in several low- and middle-income countries; booster and heterologous strategies can improve immune responses.	Neutralization and protection vary by variant, schedule and previous exposure; periodic safety/effectiveness assessment remains important.	[234, 247, 250]
Maternal vaccination	Established platforms administered during pregnancy generate maternal systemic responses and transplacental antibody transfer.	Evidence suggests protection of pregnant women from severe disease and potential infant benefits; estimates depend on circulating variants and study quality.	Current safety evidence is reassuring overall; residual confounding and representation across trimesters warrant attention.	[241]
Vaccination in immunocompromised populations	Additional doses or adapted strategies seek to overcome quantitatively and qualitatively impaired responses.	Prioritize prevention of severe disease, individualized schedules, and integration with clinical risk reduction.	Response heterogeneity is substantial; some patients remain susceptible despite recommended doses.	[136, 242, 248]
Intranasal/mucosal candidates	Mucosal delivery aims to induce local respiratory immunity in addition to systemic responses.	Phase 1 and phase 3 studies support feasibility and immunogenicity in selected settings; durable prevention of transmission requires further trials.	Long-term safety, cross-variant effectiveness, manufacturing and standardized mucosal immune endpoints remain open questions.	[255, 256]
Broadly protective sarbecovirus candidates	Mosaic RBD nanoparticles or conserved-antigen platforms target cross-reactive antibodies against multiple sarbecoviruses.	Strong preclinical proof of concept; early human trials are preliminary and do not establish broad clinical efficacy.	Need clinical correlates, breadth against future viruses, durability, and scalable manufacturing.	[252–254, 266, 267]

RBD: receptor-binding domain; mRNA: messenger RNA

Vaccine platforms and mechanisms of action

Several technological platforms were successfully deployed, including messenger RNA (mRNA), nonreplicating viral-vector, inactivated whole-virus, and recombinant protein-subunit vaccines. Lipid nanoparticle-formulated mRNA vaccines deliver nucleic acid encoding spike antigen, enabling transient antigen expression and subsequent B-cell and T-cell priming. In the pivotal mRNA-1273 trial, vaccination was associated with high efficacy against symptomatic COVID-19 in the pre-Omicron period, supporting the feasibility of rapid sequence-based vaccine development [233]. Recombinant spike-protein nanoparticle vaccines, including adjuvanted NVX-CoV2373, similarly demonstrated efficacy in randomized trials during circulation of earlier lineages [234].

Platform differences affect antigen presentation, innate immune stimulation, reactogenicity, manufacturing requirements, cold-chain needs, and suitability for repeat administration. Inactivated vaccines present a broad collection of viral antigens but may elicit different neutralizing-antibody profiles than spike-focused formulations; adenoviral vectors achieve intracellular antigen production without the use of lipid-encapsulated mRNA, whereas protein-subunit vaccines require optimized adjuvant systems. Cross-platform comparisons must account for clinical endpoints, circulating variants, immunological history, population demographics, and follow-up rather than interpreting efficacy differences between unrelated trials as direct comparative effects [235].

Vaccine immunogenicity and immune correlates

Neutralizing antibodies directed against spike, especially its receptor-binding domain, contribute to protection against acquisition of infection, while memory B cells, CD4+ helper T cells, CD8+ cytotoxic T cells, and non-neutralizing antibody functions contribute to broader and more durable immunity. Antigenic evolution can substantially reduce neutralization of heterologous variants without proportionately abolishing cellular responses. Consequently, serum binding-antibody concentrations or neutralizing titers should not be interpreted as definitive individual-level predictors of clinical protection. Analyses of the original mRNA-1273 efficacy trial identified immune markers correlated with protection, but no single universally applicable threshold has been validated across platforms, variants, clinical risk groups, and laboratory assays [101,115].

Hybrid immunity arising from vaccination and infection can broaden antigen recognition, yet its magnitude and duration depend on the sequence, timing, and antigenic diversity of exposures. High antibody titers following recent infection do not establish that further exposure is harmless, and deliberate infection is not an appropriate immunization strategy. For immunocompromised individuals, a measurable binding-antibody response may coexist with impaired functional responses, making clinical outcomes and appropriate vaccination schedules more informative than isolated serological tests [101,115].

Vaccine efficacy and real-world effectiveness

Vaccine efficacy measured in randomized trials must be distinguished from vaccine effectiveness estimated in routine clinical settings. Observational designs, particularly test-negative case-control studies, permit assessment of updated formulations after randomized placebo-controlled trials have become less feasible, but they are vulnerable to differences in testing, healthcare-seeking behavior, previous infection, underlying risk, and timing since vaccination. Estimates should specify the calendar period, comparator, target population, vaccine formulation, and outcome rather than describing a single vaccine effectiveness value as universally applicable [236,237].

In a meta-analysis of 21 studies involving more than 53 million participants, monovalent XBB.1.5 vaccination was associated with estimated first-month effectiveness of 52.9% against infection, 64.4% against COVID-19-related hospitalization, and 77.3% against death; effectiveness declined with time and differed during the subsequent JN.1 period [236]. An Influenza and Other Viruses in the Acutely Ill (IVY) Network analysis similarly documented measurable protection against hospitalization from both XBB and JN-lineage infections, although effectiveness against the latter was less certain [237]. These findings underline why endpoint selection, immune escape, and temporal waning must be evaluated jointly.

More recent data continue to support protection against severe outcomes. In a multicenter study of adults hospitalized during September 2024–April 2025, estimated effectiveness of 2024–2025 vaccines was 40% (95%CI: 27%–51%) against COVID-19-associated hospitalization and 79% (95%CI: 55%–92%) against the composite of invasive mechanical ventilation or death [238]. A Japanese test-negative study of JN.1-adapted vaccination during October 2024–April 2025 reported an effectiveness estimate of 61.8% (95%CI: 21.5%–81.4%) against hospitalization among adults aged 60 years or older [239]. Such estimates reflect defined study populations, circulating lineages, and intervals after vaccination rather than fixed vaccine properties. A 2026 systematic review likewise found that protection against infection was less durable than protection against severe disease across successive Omicron lineages [240].

Updated vaccine formulations and booster strategies

SARS-CoV-2 antigenic evolution has necessitated periodic modification of vaccine antigen composition. Variant-adapted formulations aim to narrow the antigenic distance between the vaccine immunogen and circulating viruses, while repeated doses restore waning functional antibody activity in persons at risk of serious outcomes. Neither an exclusively variant-matched nor an exclusively ancestral-strain antibody response can fully explain clinical protection because pre-existing memory and cross-reactive T-cell responses shape secondary immunity. Decisions about composition and booster timing therefore require complementary data from genomic surveillance, antigenic characterization, immunogenicity studies, and observed effectiveness against severe outcomes [236–240].

A risk-based approach is appropriate in settings where baseline population immunity is widespread and the absolute risk of severe disease is highly heterogeneous. Age, immune suppression, pregnancy, frailty, institutional residence, comorbidities, and time since the last immune exposure should inform prioritization; vaccine availability and local regulatory recommendations determine the precise product and schedule. Increasing the frequency of booster doses without assessing baseline risk may produce diminishing absolute benefit in lower-risk groups while diverting resources from poorly protected high-risk individuals. Because clinical recommendations change, a narrative review should not reproduce any national seasonal dosing schedule as globally binding [240].

Vaccination in special populations

Older adults and individuals with major chronic conditions account for a disproportionate share of severe COVID-19 outcomes, providing an important rationale for sustained access to updated vaccination. In pregnancy, both maternal vulnerability to severe respiratory illness and the potential benefit of transplacental antibody transfer must be considered. A living systematic review involving 177 studies reported findings consistent with protection against symptomatic infection and severe maternal COVID-19 without identifying a consistent increase in major maternal or neonatal adverse outcomes; the certainty of evidence was low for several outcomes, reflecting observational designs and heterogeneity [241]. Risk-benefit assessment should be individualized while avoiding the exclusion of pregnant populations from evidence-based preventive services.

Immunocompromised populations encompass heterogeneous conditions, including solid-organ transplantation, hematological malignancies, advanced immunodeficiency, and exposure to B-cell-depleting or other potent immunosuppressive therapies. Some individuals have impaired primary antibody responses, accelerated waning, or reduced immune memory despite completing a conventional vaccination schedule. A 2026 PubMed-indexed IDSA guideline supports vaccination in immunocompromised persons, with timing individualized in relation to clinical stability and immunosuppressive treatment [242]. Adjunctive prevention, vaccination of close contacts, and prompt access to diagnosis and antiviral treatment remain important because even repeated vaccination may not achieve equivalent protection in all subgroups.

Vaccine safety and adverse events following immunization

COVID-19 vaccination has an extensive accumulated safety evidence base, but clinical assessment should distinguish common transient reactogenicity, rare causally associated adverse reactions, coincidental conditions, and signals arising from biased reporting. Local pain, fatigue, myalgia,

and short-lived fever occur after multiple vaccine platforms and generally resolve without intervention. Myocarditis and pericarditis have been associated with mRNA vaccination, particularly after certain doses in adolescent boys and young men; a stratified systematic review estimated an attributable myocarditis or pericarditis risk of approximately 20.02 per 100,000 doses after mRNA-1273 in men aged 18-24 years, although estimates varied by study and case definition [243].

Vaccine-induced immune thrombotic thrombocytopenia is a rare, potentially serious syndrome recognized following some adenoviral-vector vaccines and is mechanistically distinct from routine venous thromboembolism [244]. Product-specific benefit-risk assessment must consider the individual's baseline risk of severe COVID-19, age, sex, alternative available products, past reactions, and the changing prevalence of infection. Comparisons between vaccination-associated adverse-event rates and infection-associated complications should use appropriately aligned time windows, denominators, and outcome definitions; crude passive-reporting counts cannot estimate incidence or establish causation. A 2026 methodological review of myocarditis studies highlighted substantial heterogeneity in design, follow-up windows, and control for prior SARS-CoV-2 infection [245]. Active pharmacovigilance, transparent risk communication, and accessible clinical evaluation are essential for maintaining confidence without minimizing rare adverse events.

Vaccine acceptance, hesitancy, and equitable access

Vaccine acceptance is a dynamic behavioral outcome shaped by perceived disease risk, trust in healthcare institutions, previous vaccination experiences, communication environments, religious or social concerns, practical access, and anticipated adverse effects. It should not be treated as an immutable characteristic of a population or reduced to a single deficit in knowledge. In harmonized phone surveys involving more than 120,000 respondents across 53 low- and middle-income countries during 2020-2021, approximately one in five adults reported hesitation, commonly related to vaccine safety and effectiveness [246]. These estimates describe a specific early-pandemic period and cannot establish current levels of hesitancy in 2026.

Effective communication is transparent about the principal contemporary benefit of vaccination—protection against severe outcomes—without promising complete prevention of infection or denying the existence of adverse events. Community engagement, engagement of locally trusted healthcare professionals, accessible opportunities for questions, and practical removal of cost or travel barriers can complement tailored risk communication. Programs should evaluate actual vaccination completion and protection of high-risk groups, rather than relying exclusively on stated willingness or aggregate population-level coverage [246].

The global distribution of vaccine development and deployment was markedly unequal. An analysis of worldwide introduction, scale-up, and effectiveness evaluation demonstrated later rollout and slower coverage achievements in lower-income settings, together with important evidence gaps for vaccines widely used outside high-income countries [247]. These disparities were not merely geographic: age, rural residence, economic deprivation, displacement, informal employment, and difficulties accessing adult immunization services may each affect timely vaccination. Equity-oriented prevention therefore requires attention to product availability, reliable service delivery, trusted information, functional safety monitoring, and continuity of access to diagnosis and treatment.

Pre-exposure prophylaxis and protection of highly vulnerable populations

For a subset of individuals with profound immune impairment, vaccination alone may not elicit sufficient functional protection. Pre-exposure prophylaxis with a monoclonal antibody capable of neutralizing contemporaneous variants can offer an additional protective option when clinical evidence, regulatory authorization, local availability, and susceptibility data support its use. Pemivibart has been assessed for this indication in selected immunocompromised populations. A 2025 IDSA evidence-based guideline update, published in a PubMed-indexed journal, includes a recommendation addressing pemivibart pre-exposure prophylaxis [248]. Such use should not be generalized to immunocompetent individuals or portrayed as a substitute for vaccination.

The utility of antibody prophylaxis is vulnerable to viral evolution: substitutions in the spike protein can markedly reduce neutralization by otherwise potent monoclonal antibodies.

Recommendations should therefore be reassessed as variant susceptibility and product availability change. Potential hypersensitivity reactions, infusion-related adverse effects, resource costs, and uncertainty about clinical effectiveness in distinct risk subgroups require consideration. The wider protective strategy for profoundly immunocompromised individuals should also incorporate vaccination of household contacts, mitigation of high-risk indoor exposures, prompt testing when symptomatic, and early access to clinically indicated antivirals [242,248].

Integrated surveillance and public health response

Sustainable COVID-19 prevention depends on surveillance capable of detecting changes in transmission, clinical severity, viral evolution, and the performance of preventive technologies. As routine case testing has declined and reporting methods have become heterogeneous, the number of confirmed cases increasingly reflects testing practices as well as underlying infection incidence. A resilient surveillance architecture combines sentinel respiratory illness systems, hospitalization and mortality reporting, laboratory confirmation, targeted whole-genome sequencing, wastewater surveillance, and evaluations of vaccine effectiveness and safety [30,31,55-58]. No single surveillance source provides a complete or unbiased representation of population disease burden.

Sentinel clinical surveillance provides denominator-linked information about severe outcomes and circulating respiratory pathogens, whereas wastewater monitoring can detect viral circulation without relying on individual healthcare seeking. Genomic sequencing identifies emerging lineages and potentially relevant antigenic changes, but sequence frequency alone cannot establish increased pathogenicity or vaccine escape. Interpretation requires triangulation with antigenic assays, clinical cohorts, healthcare burden, and behavioral information [30,55,57,58]. Transparent metadata, stable sampling strategies, timely reporting, and representativeness across socioeconomic and geographic strata are essential for comparing trends. The public health response should be proportionate and targeted, with particular attention to outbreaks in hospitals, residential care, and other settings involving high-risk individuals.

Monitoring vaccination programs should include product-specific coverage among priority populations, uptake of updated formulations, interval since last dose, implementation costs, and documented protection against hospitalization or death. Adverse events following immunization require both spontaneous reporting and well-designed active surveillance, together with causality assessment where appropriate. Integration of these data streams into routine public health systems can strengthen broader respiratory-virus preparedness while avoiding the unsustainable maintenance of parallel emergency structures.

Prevention strategies in low- and middle-income countries

In LMICs, sustainable prevention requires pragmatic adaptation to health-system resources rather than uncritical replication of high-income-country delivery models. Early mass vaccination programs often depended on external financing, temporary outreach arrangements, specialized reporting systems, and emergency logistics. As the epidemiology of COVID-19 evolved, incorporation of targeted vaccination into existing primary healthcare and adult-immunization services became a major implementation priority. A global survey of 17 countries described movement toward routine delivery alongside persistent financing and operational barriers [249].

Country experiences indicate that preparedness is strengthened by investment in trained vaccinators, reliable cold chains, accessible clinical registers, community partnerships, and systems capable of identifying older adults and people living with chronic disease. An evaluation of COVID-19 immunization implementation in six LMICs identified common lessons concerning planning, digital systems, workforce capacity, financing, and communication [250]. Research should examine whether integration improves timely vaccination among individuals at highest risk without displacing established maternal and childhood immunization services. In geographically dispersed settings, mobile clinics, decentralized primary care, outreach linked to chronic-disease services, and community participation may improve reach, but their comparative cost-effectiveness should be evaluated locally.

Global vaccine equity also depends on manufacturing diversification, technology transfer, predictable procurement, transparent regulatory pathways, and affordability. An analysis of COVID-19 vaccine innovation and supply models documented that access varied substantially by developer, market priorities, and product distribution strategy [251]. For countries such as Indonesia, future planning should explicitly account for the coexistence of different vaccine platforms, heterogeneity in previous infection histories, rural and island access barriers, and variable healthcare infrastructure. Policy priorities should be informed by locally measured hospitalization risk, supply chains, and program performance rather than assumptions that all populations require identical frequencies of revaccination.

Future vaccines and next-generation preventive technologies

A central limitation of current intramuscular vaccination is that robust systemic immunity does not necessarily produce durable sterilizing protection in the upper respiratory mucosa. Next-generation approaches consequently aim to improve breadth against antigenically divergent variants, extend the duration of clinically meaningful protection, and generate local mucosal immune responses capable of reducing acquisition or onward transmission. These objectives are biologically distinct and require separate clinical endpoints. Demonstration of stronger serum neutralization, greater nasal secretory immunoglobulin A, or protection in an animal-challenge model does not establish reduced human transmission without appropriately designed studies.

Broadly protective sarbecovirus vaccines seek to focus antibody responses on conserved epitopes shared by multiple SARS-related coronaviruses. Experimental multivalent receptor-binding-domain nanoparticle platforms have elicited cross-neutralizing responses and heterologous protection in animal models [252]. Mosaic nanoparticles displaying antigens from multiple sarbecoviruses protected animals against challenges involving viruses not represented directly in the vaccine formulation, supporting their potential as a platform for preparedness against future spillovers [253]. Additional studies of cocktail spike-protein nanoparticles have similarly provided preclinical evidence of cross-clade protective responses [254]. Whether these strategies provide sufficiently durable, broadly protective immunity with acceptable reactogenicity in humans remains to be established in appropriately powered trials.

Mucosal vaccination is being investigated to strengthen local immunity at the primary portal of viral entry. In a randomized phase 3 Indian trial, intranasal adenovirus-vectored BBV154 administered as a heterologous booster was evaluated for safety and immunogenicity after an intramuscular primary series; the trial did not provide definitive evidence that the intervention reduced transmission in real-world populations [255]. A small phase 1 trial of intranasal Newcastle disease virus-based NDV-HXP-S in 35 previously vaccinated adults reported acceptable short-term tolerability and modest changes in salivary secretory IgA, but it was not powered to establish protective efficacy [256]. These studies illustrate the promise and the current evidentiary limitations of the mucosal approach.

Future preventive technologies may combine multivalent immunogens, optimized delivery routes, conserved spike or non-spike antigens, novel adjuvants, and platforms supporting rapid updating and scalable manufacturing. Their value should be judged by efficacy against clinically meaningful outcomes, durability, safety in high-risk groups, manufacturability, accessibility, and the potential to mitigate viral transmission, rather than by laboratory immunogenicity alone. Coordinated clinical trial networks, standardized mucosal assays, genomic and antigenic surveillance, and equitable early access will be necessary to translate promising experimental designs into broadly useful population-level interventions.

Remaining challenges and future perspectives

Continued viral evolution and the risk of emerging variants

The continuing evolution of SARS-CoV-2 represents a central challenge for the long-term management of COVID-19. Unlike the early pandemic, during which most individuals had little pre-existing SARS-CoV-2-specific immunity, the contemporary evolutionary landscape is shaped by repeated exposures through infection and vaccination. Antigenic selection can favor viral lineages that retain sufficient replicative competence while partially escaping antibodies elicited by previously circulating strains. However, the emergence and subsequent dominance of a lineage

reflect several interacting determinants, including founder effects, contact patterns, population-level susceptibility, transmission advantages, and stochastic introduction events. Consequently, neither the number of spike mutations nor in vitro neutralization escape should be regarded as a sufficient predictor of epidemiological success [257].

The evolutionary process is further complicated by convergent acquisition of mutations, epistatic interactions among amino acid substitutions, and recombination between co-circulating lineages. Although spike evolution attracts substantial attention because of its importance for receptor engagement and antibody recognition, mutations elsewhere in the genome may influence replication, innate immune antagonism, and other aspects of viral fitness. Systematic functional studies demonstrate that individual spike substitutions can exert different effects on antibody resistance, cellular entry, and infectivity, and that contemporary variants can exhibit progressively reduced susceptibility to selected components of vaccine-induced humoral immunity [258]. Nevertheless, pseudovirus infectivity and neutralization assays capture only selected biological processes; they cannot independently establish increased clinical severity or population-level transmissibility.

Future surveillance should therefore move beyond naming lineages and reporting their relative frequencies. An integrated assessment should examine absolute viral activity, representative sequence sampling, antigenic phenotype, transmission growth relative to competing variants, evidence of diagnostic or antiviral escape, and changes in age-standardized hospitalization and mortality. Particular attention is required when interpreting early reports of unusually divergent variants, because estimates derived from small and selectively sampled populations are susceptible to bias. Evolution should not be assumed to progress predictably toward lower virulence, nor should every antigenic change be interpreted as evidence of imminent major clinical impact [55,58,257,258].

Research priorities include longitudinal mapping of antigenic distances among emergent lineages, standardized phenotypic characterization using well-defined immune sera, investigation of non-spike determinants of fitness, and rapid linkage of genomic observations to clinically meaningful outcomes. More representative sequence contributions from low- and middle-income countries are essential because geographically concentrated sampling can distort the inferred timing of emergence and global diversity of variants. Sustaining these capabilities within routine respiratory-virus surveillance, rather than relying on short-term emergency infrastructure, will determine whether newly emerging lineages are identified early enough to support timely public health decisions.

Uncertainties regarding the long-term consequences of reinfection

Repeated SARS-CoV-2 infection has become an expected feature of population-level exposure, but its cumulative health consequences remain incompletely defined. A key methodological challenge is separating the effect of an additional infection from differences in baseline health, prior exposure, vaccination history, testing intensity, and time at risk. Individuals with documented reinfections are not necessarily representative of all reinfected persons; their probability of testing, accessing care, or receiving a long COVID diagnosis may differ from that of otherwise comparable individuals with undocumented infections. In addition, estimates obtained before widespread immunity or during periods of distinct variants cannot be directly generalized to contemporary populations [29,198,259].

Prospective and registry-based investigations indicate that reinfection can be followed by persistent symptoms, although the magnitude and consistency of an additional risk compared with a single infection vary by outcome definition and study population. In the INSPIRE registry, the investigators specifically examined the relationship between reported reinfection and symptoms at three to six months, emphasizing the importance of prospective symptom ascertainment and an explicit definition of reinfection [259]. Complementary population-based analyses in Singapore evaluated new diagnoses across multiple organ systems after reinfection in different variant periods, addressing the clinical burden beyond narrowly defined symptom-based long COVID [260]. Together, these investigations strengthen the rationale for continued follow-up but do not resolve whether every subsequent infection confers an equivalent incremental risk.

A particularly important uncertainty concerns causality. Post-infection fatigue, dysautonomia, cognitive symptoms, cardiovascular events, and other conditions have multiple potential determinants, some of which are common in uninfected populations. Establishing the attributable contribution of SARS-CoV-2 requires appropriate uninfected comparators, ascertainment of pre-existing symptoms and disorders, control for healthcare-use differences, and repeated measurements of functional outcomes. Outcomes should be evaluated separately for laboratory-confirmed reinfection, clinically suspected reinfection, and exposure episodes identified through longitudinal surveillance, because these definitions select different populations. Analyses that treat all post-infection manifestations as a uniform biological entity risk obscuring distinct mechanisms and treatment-responsive phenotypes [198,203,259,260].

Future studies should prioritize prospective cohorts with repeated virological, immunological, clinical, and patient-reported measurements; explicit documentation of viral lineage and vaccination status; and sufficiently long observation to distinguish persistence, relapse, and new-onset morbidity. The absolute risk of hospitalization, organ-specific disease, functional limitation, and mortality following each infection should be presented alongside relative effects. Such evidence is necessary to quantify cumulative burden without overstating the implications of association studies or disregarding the protective contribution of existing immunity.

Gaps in understanding immune protection and immune escape

Despite advances in SARS-CoV-2 immunology, a universally applicable correlate of protection remains elusive. Circulating neutralizing antibody titers are informative population-level predictors of susceptibility, particularly for infection and symptomatic disease, but the quantitative relationship depends on the antigenic match between assay virus and circulating lineage, interval since exposure, and host characteristics. A household cohort in Nicaragua demonstrated variant-specific associations between pre-exposure neutralization titers and protection against Omicron infection, supporting use of appropriately matched assays while highlighting the limited transportability of a single antibody threshold across populations and variants [261]. Protection against severe disease additionally involves memory B cells, T-cell responses, and other host mechanisms not captured by one serological measurement.

The immunological consequences of sequential exposure are also complex. Exposure to antigenically distinct SARS-CoV-2 strains can broaden cross-neutralization; however, the initial immunizing antigen may disproportionately shape the specificity of antibodies elicited by subsequent encounters. A study comparing defined two-exposure histories found that breadth could improve without uniformly generating strong de novo responses directed specifically against the most recently encountered variant [262]. Immune imprinting should therefore not be interpreted as uniformly harmful: recalling established memory can support rapid protection, whereas a highly biased response may limit the capacity to respond optimally to antigenically distant epitopes. The clinical importance of these competing effects remains incompletely established.

Recent observations following updated LP.8.1-directed vaccination suggest that antigen-matched boosting can alter the balance between ancestral and contemporary variant neutralization. Nevertheless, studies relying on small immunogenicity cohorts, pseudovirus systems, or short-term antibody measurements cannot establish durability of protection against severe disease or transmission. Further evaluation requires standardized assays, longitudinal sampling, direct comparison of different antigenic histories, and assessment in older adults and people with impaired immune responses [263]. Studies of cellular immunity should be integrated with humoral measurements rather than conducted in isolation, particularly for outcomes involving hospitalization and death.

A related question is whether broadly protective vaccines can be designed to elicit responses against conserved coronavirus epitopes without sacrificing potency against circulating SARS-CoV-2. Evidence of cross-neutralization or protection in animal challenge models constitutes a valuable preclinical milestone, not clinical proof of broad human efficacy. Progress will depend on defining robust immunological endpoints, developing antigenic maps that account for individual exposure histories, and identifying markers that predict both the breadth and duration of clinically meaningful protection [252–254,261-263].

Challenges in antiviral and vaccine development

The therapeutic armamentarium has improved substantially, yet new treatment development faces the combined challenges of viral evolution, declining population-level event rates, limited eligibility for certain agents, and heterogeneous host responses. For antiviral drugs, susceptibility can be modified by substitutions in therapeutic targets, although detection of a putative resistance-associated mutation does not by itself demonstrate clinically important resistance. In an observational cohort with serial sequencing, treatment-emergent mutations after nirmatrelvir exposure were detected, particularly among immunosuppressed patients, but were commonly low frequency and transient; the findings did not establish widespread transmission of resistant lineages [264]. This distinction between molecular detection, phenotypic resistance, persistent viral replication, and treatment failure is fundamental for interpreting resistance surveillance.

Prolonged infection in individuals with substantial immune impairment may allow extended viral replication and selection under antiviral exposure. Characterization of remdesivir-associated polymerase substitutions in patients with immunosuppressive conditions provides additional mechanistic evidence that drug susceptibility can change during treatment, although results obtained from small, highly selected cohorts cannot establish the frequency of resistance in routine clinical practice [265]. Future studies should combine whole-genome sequencing, culture or validated measures of replication, phenotypic drug susceptibility, treatment history, and patient-centered outcomes. Combination therapy and extended treatment durations warrant controlled assessment in carefully defined populations rather than broad adoption based solely on uncontrolled case series.

Clinical trials must also address changing baseline risks. Earlier antiviral trials were often conducted before widespread vaccination and prior infection, whereas contemporary eligible populations differ in age, immune history, comorbidities, and competing risks. Demonstrating superiority when hospitalization events are uncommon may require very large samples, enrichment for high-risk patients, or carefully justified alternative endpoints. Pragmatic trials should prospectively define subgroup analyses for immune compromise, treatment initiation interval, pharmacological interactions, and variant period, while minimizing post hoc interpretation of small or unstable effects [10,11,169–197].

Vaccine development presents a complementary challenge: maximizing breadth, durability, and mucosal protection without compromising clinical efficacy, safety, or feasibility of scale-up. A 2026 first-in-human phase I study of the candidate pEVAC-PS pan-sarbecovirus vaccine established an early clinical evaluation pathway for broadly protective immunization, but initial dose-escalation data cannot establish population effectiveness against divergent sarbecoviruses [266]. A contemporaneous workshop report outlined distinct preparedness use cases and desired product characteristics for pan-sarbecovirus vaccines, emphasizing the need to define intended indications, target populations, development pathways, and equitable access before late-stage efficacy testing [267]. Progress will require adaptive trial networks, standardized immunobridging criteria, and manufacturing platforms capable of being updated without prolonged interruptions in supply.

Limitations of current surveillance and disease burden estimation

The transition away from large-scale emergency testing has introduced substantial uncertainty into the measurement of contemporary SARS-CoV-2 transmission and its clinical impact. Notification-based incidence is strongly affected by access to tests, individual testing behavior, reporting rules, and selection of symptomatic patients for testing. Hospital admission surveillance is more directly related to severe disease, but comparability can still be affected by admission thresholds, incidental detection, coding practice, and changes in the composition of the hospitalized population. Although excess mortality is a useful complementary population indicator, its interpretation requires careful consideration of disrupted healthcare, demographic change, and other causes of death [14–31].

Genomic surveillance is similarly vulnerable to sampling bias and uneven laboratory capacity. Consensus recommendations from laboratory and public health professional organizations emphasize the importance of quality-assured specimen selection, sequencing workflows, bioinformatics, metadata, and transparent reporting for meaningful comparisons across time and institutions [270]. Highly sensitive sequencing methods may identify minority

variants, but the epidemiological significance of such findings is often uncertain without information about transmission chains and population prevalence. Wastewater surveillance provides an additional, less care-seeking-dependent signal, yet differences in sewershed coverage, assay normalization, wastewater flow, and the mixture of viral lineages limit direct translation into counts of infected individuals [30,268].

Future systems should integrate sentinel respiratory testing, hospitalization surveillance, mortality monitoring, targeted genomic sequencing, wastewater measurements where feasible, and representative serological or population-based surveys. The principal objective is not maximal data collection, but a stable and interpretable set of indicators with defined denominators and explicit uncertainty. A 2026 systematic review of 363 spatiotemporal COVID-19 modeling studies identified persistent geographic concentration in high-income settings and incomplete integration of socioeconomic and fine-scale spatial information, reinforcing the need to evaluate representativeness before models are used to guide resource allocation [271]. Forecasts should report calibration and uncertainty, evaluate performance across variant transitions, and avoid presenting modeled trajectories as observations.

For low-resource and island settings, scalable surveillance may depend on strategically selected sentinel facilities, sequencing partnerships, periodic validation studies, and interoperable information systems rather than universal high-intensity testing. Public reporting should clearly differentiate infection activity, medically attended disease, hospitalizations, and deaths, and distinguish laboratory-confirmed outcomes from statistical estimates. Without such transparency, apparent changes in burden may reflect surveillance practices instead of true changes in SARS-CoV-2 transmission or pathogenicity.

Health inequities and sustainability of prevention programs

COVID-19 has exposed and amplified unequal access to prevention, diagnosis, clinical care, and protection from the social consequences of infection. Epidemiological vulnerability is not determined solely by chronological age or underlying disease; it is also shaped by occupation, crowded housing, healthcare availability, income, disability, migration status, and the resources required to act upon public health recommendations. These dimensions interact with differences in vaccine supply and delivery, producing geographic and socioeconomic disparities that cannot be resolved through biomedical innovation alone [247,250,251]. The operational challenge has shifted toward retaining sustainable protection for high-risk populations once extraordinary emergency financing and mass campaigns have ended.

Experience in the WHO South-East Asia Region illustrates the importance of adaptable laboratory networks, coordinated information systems, trained personnel, supply-chain continuity, and sustained investment in diagnostic capacity. A regional evaluation identified infrastructure and workforce constraints alongside important adaptive capacities developed during the pandemic, supporting a strategy of maintaining flexible laboratory platforms that can respond to multiple pathogens rather than SARS-CoV-2 alone [270]. In countries such as Indonesia, the costs of delivering updated COVID-19 vaccination to remote islands and populations with limited healthcare contact should be weighed against locally measured clinical risk, anticipated coverage, and opportunities for integration with existing adult and chronic-disease services.

Vaccine innovation also requires governance arrangements that promote timely and affordable access. A review of global vaccine development and distribution highlighted the mismatch between unprecedented speed of scientific development and the persistent barriers to equitable procurement, manufacturing, and delivery [271]. Prospective advance purchase mechanisms, regional production, technology transfer, transparent allocation criteria, and regulatory harmonization may improve preparedness, but each carries economic and implementation trade-offs. Equity evaluation should therefore include not only national dose availability, but also completion of recommended vaccination among clinically vulnerable groups, geographic timeliness, product suitability, and the financial consequences for households and health services.

Community participation is an additional determinant of program sustainability. A scoping review spanning several public health emergencies found that the evidence base for community-centered preparedness interventions remains heterogeneous, with much reporting concentrated

on information provision rather than substantive involvement or empowerment [272]. Recent Pacific-led experiences further illustrate the value of trusted leadership, culturally appropriate communication, and participation in institutional decision-making [273]. These lessons favor co-designed, locally evaluated approaches to vaccination and risk communication, rather than assuming that the same messages or incentives will be effective across sociocultural contexts.

The sustainability of SARS-CoV-2 prevention ultimately requires proportional allocation of resources among competing health priorities. Programs should be reassessed according to preventable severe outcomes, cost-effectiveness, operational feasibility, and unintended consequences, including potential displacement of essential routine immunization services. Explicit consideration of equity can improve the relevance and legitimacy of decisions even where evidence is incomplete, particularly when populations with the greatest clinical need are the least likely to access existing delivery platforms.

Research priorities for future coronavirus outbreaks and pandemic preparedness

The scientific response to COVID-19 demonstrated that rapid viral sequencing, platform-based vaccine development, coordinated clinical trials, and international data sharing can substantially accelerate the development of countermeasures. However, the speed of selected successes should not obscure fragmented surveillance, disparities in access, incomplete interoperability of clinical data, and the difficulty of sustaining coordinated research once acute crisis conditions subside. Future pandemic preparedness should be conceived as a continuing health-system capability rather than a collection of emergency interventions assembled after widespread transmission has begun. Strengthening routine laboratory and public health infrastructure can generate substantial benefits for influenza, respiratory syncytial virus, and other emerging infectious threats in addition to COVID-19 [268,270,271].

The most urgent research priorities are interdependent. Viral surveillance should be linked to standardized antigenic assays and estimates of clinical severity; immunological studies should define protection across different exposure histories and high-risk populations; therapeutic trials should evaluate resistance and effectiveness in contemporary settings; and post-infection cohorts should distinguish causal long-term outcomes from background morbidity. Prospective multicountry protocols with harmonized case definitions and accessible data dictionaries would enable comparisons that are currently constrained by differences in sampling and outcome ascertainment. Study designs should incorporate low- and middle-income countries from the outset rather than treating their inclusion as a later external-validation exercise [257,260,261,264,269]. The principal unresolved knowledge gaps, proposed research approaches, and expected scientific or public-health value are summarized in **Table 7**.

Preparedness for a future coronavirus with substantial pandemic potential also requires investment in broadly protective vaccine and antibody platforms, rapid diagnostics, adaptable clinical trial networks, and manufacturing arrangements that can expand production without excluding countries with limited purchasing power. Proposed vaccine development goals, including accelerated access to pan-sarbecovirus candidates, will be credible only if supported by pre-specified product profiles, validated assays, clinically meaningful trial endpoints, and transparent safety monitoring [266,267]. Shared preparedness platforms should be assessed on measurable readiness indicators, such as diagnostic turnaround, sequence representativeness, time to trial activation, workforce coverage, and equitable product delivery.

At the governance level, experience from the COVID-19 response has prompted renewed attention to transparency, accountability, financing, access to countermeasures, and fair sharing of relevant pathogen information. A 2025 scoping review examining global health governance and the WHO Pandemic Agreement highlighted persistent institutional challenges and the need to translate high-level commitments into workable implementation arrangements [274]. Future preparedness evaluations should test whether agreements and investments lead to measurable improvements in detection, research capacity, clinical protection, and equitable access rather than equating adoption of policy instruments with effective implementation.

Table 7. Current knowledge gaps and future research priorities in COVID-19

Knowledge gap	Why it remains important	Priority research approach or action	Expected scientific/public health value	References
Variant phenotyping versus clinical risk	Experimental neutralization or receptor-binding differences do not prove altered population-level severity.	Link representative sequencing, standardized virology and patient-level outcomes in prospectively analyzed cohorts.	Earlier identification of clinically important changes.	[7, 51, 52]
Durable correlates of protection	Antibody correlates for infection may not predict protection against severe disease across immune histories.	Longitudinal B-/T-cell studies with variant-matched assays and breakthrough outcomes.	Better vaccine evaluation and risk stratification.	[114, 115, 261]
Immune imprinting and breadth	Previous exposure can alter response to new antigens; the magnitude and durability of effects vary.	Randomized antigen-composition comparisons and longitudinal neutralization mapping.	More rational updated vaccine design.	[113, 262, 263]
Consequences of repeated infection	Risks of repeated acute infection and new postacute symptoms are incompletely separated from underlying health differences.	Population-based prospective studies with preinfection phenotyping and appropriate comparators.	Reliable estimates of cumulative health burden.	[206, 259, 260]
Biologically defined long COVID	No single validated diagnostic biomarker or universal mechanism explains heterogeneous phenotypes.	Mechanistic multicenter cohorts with replicated biomarkers, tissue data where ethical, and standardized case definitions.	Mechanism-linked diagnosis and targeted treatment trials.	[198, 209–213]
Disease-modifying long-COVID therapies	Current clinical care largely targets symptoms; therapeutic efficacy evidence remains inconsistent.	Adaptive randomized trials with prespecified phenotypes, patient-centered outcomes and longer follow-up.	Evidence-based rehabilitation and therapeutics.	[223–225]
Antiviral efficacy in highly immune populations	Baseline risk has changed since pivotal trials, and some low-risk groups show smaller absolute benefit.	Pragmatic randomized and well-controlled comparative studies by age, frailty and vaccine history.	More efficient risk-based treatment allocation.	[169, 172, 176]
Persistent infection and drug resistance	Prolonged replication may permit resistance in some immunocompromised patients.	Genotype–phenotype resistance networks, antiviral sequencing and treatment-combination trials.	Preserved therapeutic activity and safer prolonged therapy.	[189, 190, 264, 265]
Special populations and pregnancy	Trial exclusion, small samples and variable immune competence limit precise benefit-risk estimates.	Targeted trials, registries and linked maternal/infant and transplant outcomes.	Equitable, individualized clinical guidance.	[135, 191, 241, 242]
Next-generation mucosal vaccines	Systemic vaccination incompletely blocks respiratory infection and onward transmission.	Randomized effectiveness trials with nasal immunology and infection/transmission endpoints.	Potential for stronger infection prevention.	[255, 256]
Pan-sarbecovirus vaccines	Future coronavirus threats may not match currently circulating SARS-CoV-2.	Progress from animal challenge models to phase 2/3 trials with conserved antigen mapping and cross-protection endpoints.	Broader preparedness against antigenically diverse viruses.	[252–254, 266, 267]
Representative contemporary surveillance	Declining routine testing makes case counts less comparable across time and location.	Integrate sentinel testing, inpatient severity monitoring, wastewater and calibrated serology.	Less biased and timelier burden estimates.	[14, 30, 31, 269]
Global genomic surveillance equity	Genome sampling is geographically uneven and can miss emerging lineages.	Fund regional sequencing networks, transparent standards and equitable data sharing.	Improved early-warning capacity.	[55, 57, 58, 268]
Implementation and vaccine inequity	Delivery, trust, financing, and supply constraints disproportionately affect some populations.	Implementation-effectiveness studies and community co-designed, context-specific programs in LMICs.	Sustainable access and reduced preventable burden.	[246, 247, 249, 250]
Pandemic-ready trial infrastructure	Traditional single-center studies cannot rapidly answer urgent questions during new epidemic waves.	Maintain adaptive platform trials, harmonized endpoints and international data-governance agreements.	Faster generation of actionable evidence.	[170, 271, 272, 274]

LMICs: low- and middle-income countries

The long-term objective should be an integrated and adaptable system capable of detecting biologically important changes, interpreting their clinical relevance, protecting those at greatest risk, and generating rigorous evidence under conditions of uncertainty. Such a system depends on sustained collaboration between virologists, immunologists, clinicians, epidemiologists, implementation researchers, policymakers, and affected communities. Continued SARS-CoV-2 circulation provides an opportunity to strengthen these capacities while reducing the persistent burden of COVID-19 and improving readiness for future coronavirus emergencies.

Conclusion

COVID-19 continues to represent a significant global health challenge, even beyond its acute pandemic phase, due to the ongoing evolution of SARS-CoV-2, changing population immunity, recurrent infections, and the persistent consequences of post-COVID-19 condition. Although substantial advances have been achieved in understanding viral biology, transmission, pathogenesis, diagnosis, vaccination, and therapeutic management, important challenges remain in addressing immune escape, reinfection, long-term complications, and disparities in access to healthcare. The evolving epidemiological landscape also highlights the importance of strengthening surveillance systems, maintaining evidence-based preventive and clinical interventions, and improving the recognition and management of vulnerable populations. Future efforts should prioritize sustained research, equitable access to vaccines and treatments, and international collaboration to address remaining scientific uncertainties and emerging public health needs. An integrated and adaptable approach will be essential not only to mitigate the continuing burden of COVID-19 but also to strengthen global preparedness and resilience against future infectious disease threats.

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Declaration of artificial intelligence use

ChatGPT Plus (OpenAI, San Francisco, CA, USA) was used to assist with manuscript writing, language refinement, and formatting. All AI-assisted content was reviewed and verified by the authors, who take full responsibility for the accuracy, integrity, and final content of the manuscript.

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