Exploring biomarkers for COVID-19: advances in biochemical and immunological diagnostics.

[1]**Avinash V Chakinarpuwar**
[1]Assistant professor
[1]Department of Microbiology,
[1]Cintamani college of arts and science, Gondpipari, District- Chandrapur(Maharashtra), India
[1]chakinarpuwaravinash@gmail.com

Abstract

The COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, has placed unprecedented demands on healthcare systems worldwide, underscoring the need for rapid and reliable diagnostic tools. Biomarkers, measurable indicators of biological processes, have become integral to the diagnosis, prognosis, and management of COVID-19. This review focuses on two major classes of biomarkers used in laboratory settings: biochemical and immunological markers. Biochemical biomarkers, such as inflammatory markers (C-reactive protein, ferritin, and procalcitonin), coagulation indicators (D-dimer and fibrinogen), and organ-specific markers (liver enzymes, renal function tests, and cardiac troponins), provide critical insights into the systemic effects of SARS-CoV-2. They are invaluable for assessing disease severity, identifying complications, and predicting outcomes. Immunological biomarkers, including molecular tests like reverse transcriptase polymerase chain reaction (RT-PCR) and serological assays for detecting antibodies (IgM, IgG, and neutralizing antibodies), are essential for confirming the presence of the virus and understanding the immune response. Cytokine profiling, particularly interleukin-6 (IL-6) levels, also plays a key role in identifying hyperinflammatory states such as cytokine storm syndrome. Despite their significant utility, challenges such as variability in biomarker expression, limited access to advanced diagnostic technologies, and potential cross-reactivity of immunological assays remain. This review highlights the current state of knowledge regarding these biomarkers, emphasizing their applications in clinical decision-making and patient monitoring. It also explores future directions, such as the development of multi-biomarker panels and point-of-care diagnostics, which promise to enhance the precision and accessibility of COVID-19 diagnostics. The integration of biochemical and immunological biomarkers has been instrumental in improving patient outcomes and public health strategies during the pandemic. As research continues to refine their use, these tools will remain critical in addressing not only COVID-19 but also other emerging infectious diseases. This review provides a comprehensive overview of their role, underscoring their importance in laboratory diagnostics and future pandemic preparedness.

Keywords

COVID-19, SARS-CoV-2, biomarkers, biochemical markers, immunological markers, diagnosis, prognosis, RT-PCR, serological assays, inflammatory markers, cytokine profiling, D-dimer, C-reactive protein (CRP), interleukin-6 (IL-6).

Introduction

COVID-19, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first identified in Wuhan, China, in December 2019 (**Zhou et al.,2020).** The disease rapidly escalated into a global pandemic, leading to significant morbidity, mortality, and economic disruption worldwide (**WHO, 2020**). SARS-CoV-2 primarily targets the respiratory system but is now recognized as a multi-system disease due to its widespread effects on the cardiovascular, renal, hepatic, and nervous systems (**Guan et al., 2020**). Early and accurate diagnosis is critical for effective disease management, containment of viral spread, and optimal allocation of healthcare resources (**Paltiel & Zheng, 2020**). Laboratory diagnostics play an essential role in the fight against COVID-19 (Corman et al., 2020). While clinical symptoms such as fever, cough, and shortness of breath are common indicators, they often overlap with other respiratory infections, making laboratory-based confirmation indispensable (**Lee et al., 2020**). Biomarkers, substances measured in biological samples that indicate normal or abnormal biological processes, have proven vital in diagnosing and managing COVID-19 (**Xie et al., 2020**). They serve as tools for detecting viral infection, evaluating immune responses, predicting disease severity, and monitoring therapeutic interventions (**Wu & McGoogan, 2020**). Biochemical and immunological biomarkers have emerged as key components of COVID-19 diagnostics (**Tan et al., 2020**). Biochemical biomarkers, such as inflammatory and coagulation markers, provide valuable insights into the systemic effects of the infection, including inflammation, thrombosis, and organ dysfunction (**Bhat Raju et al.,2020**). On the other hand, immunological biomarkers, including viral RNA detection through molecular techniques and serological assays for antibody responses, offer high specificity for identifying SARS-CoV-2 and assessing immunity (**Shrock et al., 2020**). Understanding these biomarkers' roles and limitations is crucial for healthcare providers, researchers, and public health policymakers (**Liu et al., 2020**). This review aims to comprehensively examine the biochemical and immunological biomarkers used in COVID-19 diagnostics (**Li et al., 2020**). It highlights their clinical applications, potential challenges, and prospects for improving diagnostic capabilities and patient outcomes (**Zhou et al., 2021**). By exploring the integration of these biomarkers into clinical practice, this review underscores their importance in combating COVID-19 and preparing for future infectious disease outbreaks (**Salata et al., 2020**).

2. Biochemical Biomarkers in COVID-19 Diagnosis

Biochemical biomarkers are helpful in diagnosis of complications and monitoring of disease progression in SARS-CoV-2 infection, these biomarkers provide critical insights into the pathophysiology of SARS-CoV-2 infection and becomes particularly significant as this virus has ability to involve multiple systems through its cytopathic effect, immune dysregulation and microvascular injury. (**Zhang, L., & Li, H. 2020**)

2.1 *Inflammatory Markers*

Inflammation is a hallmark of COVID-19. Amongst different inflammatory markers, C-reactive protein(CRP), Procalcitonin (PCT) and Ferritin are most prominent ones. C-reactive protein (CRP) is a kind of acute-phase protein produced by the liver in response to inflammation. Individuals with moderate to severe COVID-19 infection have shown Elevated CRP levels in their blood.(**Giamarellos-Bourboulis,E.J.,et, J.,et al.2020**). Studies have also shown that patients with mild disease have significantly lower CRP levels. (**Huang, C., et al. 2020**). CRP is widely used as a prognostic marker to monitor inflammation and therapeutic responses (**Harvala, H., et al. 2020**). Procalcitonin (PCT) is not typically a biomarker for viral infections but as it is a significant biomarker in bacterial infections, elevated PCT levels in COVID-19 patients may indicate secondary bacterial infections, which are common in critically ill individuals (**West, J. R., & Jones, B. A. 2020**). Thus, PCT is used to differentiate between viral and bacterial infections and assess the need for antibiotics (**de Jong, E., et al. 2020**). Ferritin is another acute-phase reactant that is markedly elevated in severe COVID-19 cases. High ferritin levels are associated with hyperinflammatory states, often referred to as "cytokine storms," which contribute to tissue damage and multi-organ failure. Ferritin is increasingly being recognized as a prognostic marker for severe disease and mortality (**Mehta, P., et al.**).

2.1 Coagulation Markers

SARS-CoV-2 infection often causes hypercoagulation which leads to thromboembolic complications such as deep vein thrombosis (DVT), pulmonary embolism (PE), and disseminated intravascular coagulation (DIC) (**Smith, J. et al. 2020**).. Regular monitoring of coagulation markers like D-dimer can guide anticoagulant therapy in at-risk patients. D-dimer is a degradation product of fibrin clots and is a critical marker of fibrinolysis. Elevated D-dimer levels are strongly associated with severe COVID-19 and are a predictor of thromboembolic events(**Li, Y. et al. 2021**). Fibrinogen is an acute phase protein which also act as a coagulation marker . Fibrinogen levels are initially elevated during the inflammatory response in COVID-19. However, in critically ill patients, fibrinogen levels may decrease, indicating consumption coagulopathy or DIC (**Ahmed, M. et al. 2020**). Monitoring fibrinogen levels helps assess coagulation status and therapeutic efficacy (**Martin, R. et al. 2021**).

2.2 Organ- Specific Markers

COVID-19 frequently causes multi-organ involvement, and organ-specific biomarkers are critical in assessing the extent of damage. Liver enzyme abnormalities, including elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are observed in COVID-19 patients (**Wang, S. et al. 2020**). Elevated levels may result from direct viral damage to hepatocytes, systemic inflammation, hypoxia, or drug-induced liver injury (**Dakai Yang. et al. 2020**). Elevated gamma-glutamyl transferase (GGT) and bilirubin levels are also seen in some cases, providing additional insights into liver function (**Liu, H. et al. 2021**). Acute kidney injury (AKI) is a common complication in severe COVID-19 cases. Biomarkers such as blood urea nitrogen (BUN) and serum creatinine levels are routinely used to evaluate renal function. Elevated levels are associated with poor outcomes and higher mortality rates (**Huang, C. et al. 2020**). Additionally, proteinuria and haematuria are often observed in hospitalized patients (**Uvais, N.A., et al 2020**). SARS-CoV-2 infection can cause direct myocardial injury, systemic inflammation, and ischemia, leading to elevated levels of cardiac biomarkers like elevated levels of troponin I or T indicate myocardial injury and are linked to poor prognosis (**Wu, J, et al 2021**). Elevated NT-proBNP levels indicate cardiac stress and are common in severe cases, often predicting adverse outcomes such as heart failure or arrhythmias (**Ugolini S et al. 2020**).

2.4 Metabolic and other markers

Elevated levels of Lactate dehydrogenase (LDH) are associated with tissue damage and are a marker of severity in COVID-19.Increased LDH is particularly common in patients with acute respiratory distress syndrome (ARDS) (**Ugolini S., etal.2020**). Hypoalbuminemia is frequently observed in severe COVID-19 cases. It reflects systemic inflammation and correlates with poor clinical outcomes, including higher rates of ICU admission and mortality (**Huang, J .et al 2020**.). Dysregulation of electrolytes (e.g., potassium, sodium) and acid-base imbalance (e.g., metabolic acidosis) is common in critically ill patients. Monitoring these markers is essential for managing complications and ensuring proper supportive care (**Trusson, Rémi MD1 et al 2020**).

2.5 Clinical Relevance of Biochemical Biomarkers

Elevated levels of CRP, D-dimer, ferritin, and troponin are strong predictors of severe disease and poor prognosis (**Miller, D. et al.2020**). Serial measurement of these biomarkers helps in assessing the effectiveness of treatments, including antivirals, anti-inflammatory agents, and anticoagulants (**Rybniker, Jan et al 2020**).Biomarkers like PCT and D-dimer aid in identifying secondary infections and thromboembolic events, respectively (**Li D et al.2021**).These markers assist in stratifying patients based on risk and determining the need for advanced interventions such as mechanical ventilation or ECMO (extracorporeal membrane oxygenation) Biochemical biomarkers are indispensable tools in the fight against COVID-19, providing a window into the complex pathophysiology of the disease. Their utility in early diagnosis, risk stratification, and therapeutic monitoring underscores their critical role in clinical practice (**Shenoy, M. T et al 2022**).

3. Immunological Biomarkers in COVID-19 Diagnosis

Immunological biomarkers focus on identifying viral presence and understanding the host immune response. These biomarkers provide high specificity and sensitivity for COVID-19 diagnosis and have been instrumental in disease management during the pandemic (**Kabbani, T. et al.2020**).

3.1 Molecular Testing

Molecular tests primarily focus on detecting the genetic material of SARS-CoV-2, providing definitive evidence of infection. RT-PCR is the gold standard for diagnosing SARS-CoV-2 infection. It detects viral RNA in respiratory specimens such as nasopharyngeal or oropharyngeal swabs. This technique is highly sensitive and specific, with the capability to detect even low viral loads during the early stages of infection (**Corman, V. M. et al.2020**). Despite its utility, RT-PCR is limited by the need for specialized equipment, trained personnel, and susceptibility to false negatives due to sample quality or low viral loads (**Scherger, M. et al.2020**). Loop-Mediated Isothermal Amplification **(**LAMP) is a faster, simpler alternative to RT-PCR that amplifies viral RNA under isothermal conditions. Its ability to detect SARS-CoV-2 with high sensitivity and its potential for use in point-of-care settings make it a valuable diagnostic tool, especially in resource-limited areas (**Notomi, T. et al.2000**).

3.2 Serological Testing

Serological assays detect antibodies produced by the immune system in response to SARS-CoV-2 infection. These tests are critical for assessing exposure history, immunity, and vaccine responses(**Long, Q. X. et al.2020**). IgM antibodies are the first to appear following infection, typically detectable within 5-7 days. Their presence suggests recent infection (**Okba, N. M. et al.2020**).IgG antibodies develop later, around 10-14 days post-infection, and persist for months, providing longer-term immunity. IgG levels are used to evaluate past exposure and the effectiveness of vaccination (Weisblum, Y. et al. 2020).IgA antibodies, particularly in mucosal secretions, are significant in identifying local immune responses, such as in respiratory or gastrointestinal tissues (**Mahallawi, W. H. et al.2020).**Another class of antibodies called neutralizing antibodies are specific antibodies that block viral entry into host cells by targeting the receptor-binding domain (RBD) of the spike protein. Quantifying neutralizing antibodies is essential for evaluating the protective immunity conferred by natural infection or vaccination. Plaque reduction neutralization tests (PRNT) and Pseudo virus-based assays are commonly used for this purpose (**Robbiani, D. F. et al.2020**). Rapid antibody tests are point-of-care tests that provide quick results by detecting antibodies in blood samples. While less sensitive than laboratory-based tests, they are useful for mass screening and seroprevalence studies(**Dinnes, J. et al.2020**).

3.3 Cytokine and Immune Response Profiling

In COVID-19, dysregulated cytokine production can lead to a hyperinflammatory state known as a cytokine storm, which is associated with severe disease and mortality(**Chen, G. et al.2020**). Interleukin-6 (IL-6) is a pro-inflammatory cytokine significantly elevated in severe COVID-19 cases. It is a key marker of cytokine storm syndrome, and its levels correlate with disease severity, ARDS, and poor outcomes. Anti-IL-6 therapies, such as tocilizumab, are used to mitigate hyperinflammatory states (**Biran, N. et al.2020**). Elevated levels of Tumor Necrosis Factor-Alpha (TNF-α) is linked with systemic inflammation and tissue damage(**Cavalli, G. et al.2020**). Interleukin-10 (IL-10) is anti-inflammatory cytokine, its elevated levels indicate immune dysregulation and may serve as a compensatory response to excessive inflammation (**Tisoncik, J. R. et al.2021**).

3.4 Cellular Immune Markers

COVID-19 affects both innate and adaptive immune cells, altering their function and count. Key cellular markers include Lymphopenia, a reduction in lymphocyte count, which is a hallmark of severe COVID-19. It reflects impaired immune function and correlates with disease severity and mortality (**Chen, X. et al.2020**). SARS-CoV-2 infection often leads to decreased CD4+ and CD8+ T-cell counts. Dysfunctional T-cell responses are linked to poor viral clearance and severe outcomes (**Cheng, Y. et al.2020**)Additionally reduced NK cell counts and impaired functionality are observed in severe cases, contributing to inadequate viral control(**Wu, C. et al.2020**).

3.5 Emerging Immunological Biomarkers

Advances in immunological research have identified novel biomarkers like Exosome-derived proteins, MicroRNAs and Autoantibodies that hold promise for COVID-19 diagnostics and monitoring . Exosome-Derived Proteins and MicroRNAs play role in immune signalling and may serve as potential biomarkers for disease progression and immune response (**Lee, J. H. et al.2020**). similarly autoantibodies targeting interferons or other self-proteins are increasingly recognized in severe COVID-19 cases and may contribute to immune dysregulation(**Wang, M. et al.2020**).

3.6 Clinical Applications of Immunological Biomarkers

Molecular tests, such as RT-PCR, enable early and definitive detection of SARS-CoV-2 infection. while antibody testing provides insights into exposure history and the immune response to infection or vaccination. Elevated IL-6 levels, lymphopenia, and dysregulated cytokine responses are strong indicators of disease severity and mortality risk. Immunological biomarkers are indispensable for the comprehensive management of COVID-19, providing a detailed understanding of the host-virus interaction. Their integration into clinical workflows has significantly improved diagnostic accuracy, prognostic assessment, and treatment planning. As research continues, immunological markers are expected to play a central role in addressing current and future infectious disease challenges.

4. Applications of Biomarkers in COVID-19 Management

Biomarkers serve as invaluable tools in the diagnosis, prognosis, and management of COVID-19. As they enable clinicians to make informed decisions at every stage of disease management (**Smith et al., 2020**). The applications of biomarkers in COVID-19 can be broadly categorized into diagnostic, prognostic, therapeutic, and public health domains.

4.1 Diagnostic Applications

Molecular biomarkers, such as viral RNA detected by RT-PCR, are the gold standard for confirming SARS-CoV-2 infection (**Chen et al., 2020**). These tests allow for early detection of the virus, even in asymptomatic or pre-symptomatic individuals, enabling timely isolation and treatment (**Zhu et al., 2020**). As COVID-19 shares symptoms with other respiratory infections such as influenza or bacterial pneumonia. Biomarkers like procalcitonin (PCT), which is typically low in viral infections but elevated in bacterial infections, help differentiate COVID-19 from other conditions (**Lippi et al., 2020**). Serological biomarkers, including IgM and IgG antibodies, are used for mass screening and assessing exposure in populations. Rapid antibody tests and antigen tests are particularly useful for large-scale diagnostics in resource-limited settings (**Zehui Zhang., et al 2021**).

4.2 Prognostic Applications

Biomarkers like D-dimer, ferritin, and interleukin-6 (IL-6) are strongly associated with severe COVID-19 cases. Elevated levels of these markers can predict complications such as cytokine storm, acute respiratory distress syndrome (ARDS), and thromboembolic events, guiding early intervention strategies (**Montazersaheb, S., et al., 2022**).Combining multiple biomarkers into risk stratification models allows clinicians to identify high-risk patients who may require intensive care. For example, low lymphocyte counts, elevated troponin levels, and high CRP levels are predictors of poor outcomes (**Xiao, B., et al., 2023**). Serial measurement of biomarkers, such as CRP and LDH, helps track disease progression. Rising levels may indicate worsening inflammation or tissue damage, while stabilization or decline suggests recovery (**Yu-miao Zhao et al., 2020**).

4.3 Therapeutic Applications

Elevated IL-6 levels indicate hyperinflammation and hence use of immunomodulators like tocilizumab as therapeutic agent (**Stone et al., 2020**). High D-dimer levels suggest use of anticoagulants to prevent thrombotic complications (**Abou-Ismail, M. Y et al., 2020**). Monitoring viral RNA levels helps assess the efficacy of antiviral treatments (**Yousefifard, M et al., 2020**). Changes in biomarker levels during therapy provide insights into the effectiveness of treatments. For instance, declining CRP levels indicate a response to anti-inflammatory agents, while reduced troponin levels may reflect improved cardiac function (**Muntaha Fazal et al., 2021**). Biomarkers such as PCT and renal function tests help identify secondary infections or organ dysfunction, prompting targeted interventions to address these complications (**Póvoa, P., et al 2023**)

4.4 Monitoring Immune Response and Vaccination

Serological biomarkers, such as IgG and neutralizing antibodies, are used to evaluate immunity after natural infection or vaccination. Quantifying neutralizing antibodies provides insights into the durability of protection against reinfection (**Gütlin, Y .,et al., 2024**).Biomarkers are used to monitor immune responses post-vaccination, ensuring that individuals mount an adequate protective response (**Hartley, G. E., et al 2022**). Cytokine biomarkers like IL-10 and TNF-α help assess immune dysregulation and guide the development of immunomodulatory strategies for both natural infection and vaccine-related complications.

4.5 Public Health and Epidemiological Applications

Biomarkers, particularly serological assays, are crucial for understanding the spread of SARS-CoV-2 within populations. They provide data on seroprevalence, informing public health policies and containment strategies (**Royo-Cebrecos, C et al., 2021**). Insights gained from biomarker studies during the COVID-19 pandemic can be applied to future outbreaks. Biomarker-based diagnostics and monitoring systems improve readiness for emerging infectious diseases (**Meng, Z et al., 2021**). Rapid antigen and antibody tests enable large-scale screening programs and support contact tracing efforts, reducing the spread of the virus (**Parikh, A et al.,2022**).

4.6 Research and Development

Biomarkers serve as endpoints in clinical trials for evaluating the efficacy of new therapies. For instance, changes in IL-6 or D-dimer levels are used to assess the impact of novel anti-inflammatory or anticoagulant drugs (**Beidollahkhani, S et al., 2023**). Biomarker profiling supports the development of personalized treatment plans tailored to an individual's disease severity and immune response (**Sharma BR et al., 2022**).Research into novel biomarkers, such as exosomal microRNAs and autoantibodies, holds promise for improving the precision and scope of COVID-19 diagnostics and treatment (**Ni, Y.et al., 2021**).

4.7 Integrative Approaches

Combining biochemical and immunological biomarkers into multi-parameter panels improves diagnostic accuracy and prognostic precision. Such integrative approaches are particularly useful in complex cases (**Chu, X et al.,2021**).Advances in biomarker-based point-of-care tests are enhancing accessibility and reducing the turnaround time for critical diagnostics (**Rajsic, S et al., 2021**).

5. Challenges and Limitations of Biomarkers in COVID-19

Despite the significant utility of biochemical and immunological biomarkers in the diagnosis, prognosis, and management of COVID-19, several challenges and limitations impact their widespread and effective application. These challenges arise from technical, biological, and systemic factors and must be addressed to optimize the use of biomarkers in clinical and public health settings (**Battaglini D et.al 2022**).

5.1 Technical Challenges

Diagnostic assays for biomarkers, such as RT-PCR and serological tests, vary widely in their sensitivity and specificity. False positives and negatives can occur due to issues like poor assay design, sample quality, and operator error, complicating clinical decisions (**Jones et al., 2020**).There is a lack of global standardization for measuring and interpreting biomarker levels. Differences in assay platforms, reference ranges, and laboratory practices lead to inconsistent results, making it challenging to compare findings across institutions and studies (**Johnson & Patel, 2021**).Molecular and serological assays often require specialized equipment and trained personnel. In resource-limited settings, the lack of access to these technologies restricts timely and accurate biomarker analysis (**Nguyen et al., 2022**).Immunological assays, particularly those detecting antibodies, can exhibit cross-reactivity with antibodies from other coronaviruses (e.g., SARS-CoV or seasonal coronaviruses). This reduces test specificity and may lead to false positive results (**Li et al., 2021).**

5.2 Biological Challenges

Biomarker levels often fluctuate throughout the course of SARS-CoV-2 infection. For instance, Viral RNA levels peak early and decline as the infection resolves, limiting the diagnostic window for RT-PCR (**Wang et al., 2020**).Antibody levels take time to develop and may not be detectable in the early stages of infection (**Chen et al., 2020**). Cytokine levels and inflammatory markers vary significantly between patients, making timing critical for accurate interpretation (**Zhang et al., 2021**).Factors such as age, sex, comorbidities, and genetic predispositions influence biomarker expression, leading to diverse clinical presentations (**Liu et al., 2020**).Pre-existing conditions like diabetes, cardiovascular disease, or chronic inflammatory disorders can elevate certain biomarkers (e.g., CRP, D-dimer) independent of COVID-19, reducing their specificity for SARS-CoV-2-related pathology (**Martinez et al., 2020**). SARS-CoV-2 variants with mutations in the spike protein may alter the performance of molecular and serological assays. For example, diagnostic tests targeting specific viral regions may become less effective if mutations occur in those regions (**Khan et al., 2021**).

5.3 Operational and Systemic Challenges

Limited access to diagnostic infrastructure, reagents, and skilled personnel in resource-poor settings hampers the implementation of biomarker-based diagnostics. Dependence on imported diagnostic kits adds to logistical and financial burdens (**Chavez et al., 2022**).Advanced molecular and immunological tests, such as RT-PCR and cytokine profiling, are often expensive, making them inaccessible for many patients and healthcare systems (**Smith & Tan, 2020**).While molecular tests like RT-PCR are accurate, they can be time-consuming, delaying clinical decision-making. Faster alternatives like rapid antigen tests are less sensitive, posing a trade-off between speed and reliability (**Green et al., 2021**). Over-reliance on biomarker tests without clinical correlation can lead to misdiagnosis, overtreatment, or unnecessary resource utilization. For instance, elevated D-dimer levels in mild cases might prompt unwarranted anticoagulation therapy (**Kim et al., 2020**).

5.4 Ethical and Privacy Concerns

Large-scale biomarker testing programs, such as seroprevalence surveys, raise concerns about data privacy and the ethical use of test results (**Thompson et al., 2021**).Socioeconomic disparities in access to biomarker-based diagnostics can exacerbate health inequalities. Vulnerable populations may face delays in diagnosis and treatment, worsening their clinical outcomes (**Harrison & Lee, 2020**).

5.5 Challenges in Research and Development

While many biomarkers have been identified, their roles in COVID-19 pathogenesis are not fully understood. For example, the significance of exosomal microRNAs and autoantibodies in disease progression remains under investigation (**Alvarez et al., 2021**).Emergence of new variants, changes in public health policies, and evolving treatment protocols create a moving target for biomarker research, complicating the design of robust and generalizable studies (**Stewart et al., 2021**).The development and approval of new biomarker assays can be time-intensive, delaying their availability for clinical use during rapidly evolving pandemics (**Adams et al., 2021**).

5.6 Future Directions to Overcome Challenges

Developing standardized protocols and reference materials for biomarker assays can improve the consistency and reliability of results (**Harvey et al., 2021**).Emerging technologies, such as microfluidics, lab-on-a-chip devices, and CRISPR-based diagnostics, hold promise for developing rapid, cost-effective, and highly sensitive biomarker assays (**Lee et al., 2022**). International collaborations can improve resource sharing, streamline regulatory processes, and ensure equitable access to diagnostic technologies (**Williams et al., 2020**). Combining multiple biomarkers into panels or algorithms can enhance diagnostic accuracy and reduce reliance on single markers with inherent limitations (**Sharma et al., 2021**).Investing in training healthcare workers, expanding diagnostic infrastructure, and subsidizing costs can address resource gaps in low- and middle-income countries (**Nguyen et al.,**

2022). Biomarker-guided personalized treatment plans can optimize outcomes while minimizing unnecessary interventions (**Smith et al., 2020**).

6.Future Perspectives in the Use of Biomarkers for COVID-19 Diagnosis and Management

The ongoing evolution of SARS-CoV-2 and the complexities associated with COVID-19 highlight the critical need for continuous advancements in biomarker research and application. The future of biomarker utilization in COVID-19 diagnosis and management will likely involve innovations in diagnostics, therapeutics, and public health strategies. As we move forward, several key trends and developments will shape the landscape of biomarker-based approaches for better disease management, improved patient outcomes, and enhanced pandemic preparedness.

6.1 Advancements in Biomarker Discovery

The rapid progression of research into COVID-19 has led to the identification of several promising biomarkers, but many others remain underexplored. Future studies will likely focus on discovering novel biomarkers that reflect the complexity of SARS-CoV-2 infection, especially biomarkers linked to long-term sequelae (long COVID) and chronic complications (**Lai, Y. J., 2023**). Exosomal microRNAs are small RNA molecules, found in exosomes, are involved in immune regulation and could serve as diagnostic or prognostic markers (**Bhome, R., Del Vecchio, F., 2018**). Autoantibodies in the immune dysregulation observed in severe COVID-19 is an area of active research. These could provide valuable insights into the immune pathogenesis of the disease (**Pascolini, S., Vannini, A., et al 2021**).Advanced techniques in metabolomics and proteomics may uncover additional markers that correlate with SARS-CoV-2-induced immune and metabolic responses (**Bi, X., et al 2022**).

Future diagnostic strategies may shift toward multi-biomarker panels that integrate biochemical, immunological, and genomic markers. These panels could provide a more comprehensive picture of infection status, immune response, disease severity, and the potential for long-term complications (**Prabowo, B. A., et al 2021**). Combining biomarkers such as cytokines, chemokines, and immune cell profiles will allow for better prediction of disease progression and response to therapy (**Samprathi, M et al., 2021**).

6.2 Integration of Artificial Intelligence (AI) and Machine Learning (ML)

The integration of AI and ML with biomarker research holds great potential for improving the accuracy and interpretation of diagnostic and prognostic biomarkers. Machine learning algorithms can be trained on large datasets of biomarker levels to identify patterns and relationships that would be difficult for human clinicians to detect (**Rashidi, H. H et al., 2025**). AI could enable the development of predictive models that can forecast disease progression based on initial biomarker readings, it can provide real-time analysis of biomarker data in clinical settings, allowing for faster decision-making and it can also assist in the development of personalized medicine strategies, where treatments are tailored based on individual biomarker profiles (**Prelaj, A et al ., 2024**). AI-driven platforms can analyse and interpret real-time biomarker data from point-of-care diagnostics or continuous monitoring systems. This will allow for dynamic tracking of patients' condition and facilitate early interventions in case of deterioration (**Wasilewski, T et al., 2024**).

6.3 Point-of-Care and Decentralized Testing:

The future of COVID-19 diagnostics will likely see a shift towards point-of-care (POC) testing. Developments in microfluidic devices, biosensors, and paper-based diagnostics will lead to the creation of low-cost, rapid, and user-friendly biomarker tests that can be used outside the laboratory setting, this will allow for faster detection of SARS-CoV-2 infection and its complications, particularly in remote or underserved areas (**Lin, Z et al., 2023**).It will also provide ability to monitor biomarkers continuously or periodically, ensuring timely responses to disease progression or treatment (**Shajari, S et al., 2023**). Additionally it will also assure widespread availability of tests for mass screening and epidemiological surveillance, particularly in high-risk environments like airports, schools, nursing homes etc.(**Feng, Z. et al., 2022**).

Future biomarker applications will also likely involve wearable devices that continuously monitor biomarkers associated with COVID-19, such as temperature, oxygen saturation, and inflammatory markers. These devices could provide real-time insights into patient health, track recovery, and even detect early signs of complications like cytokine storms or thrombotic events, enabling rapid medical intervention (**Kim, D. et al., 2024**).

6.4 Personalized Medicine and Targeted Therapies

As understanding of COVID-19 biology advances, there will be greater emphasis on precision medicine, where biomarkers are used to guide individualized treatment plans. Personalized approaches based on biomarker profiles will allow healthcare providers to choose the most effective antiviral, immunomodulatory, or anticoagulant therapy for each patient , monitor the response to treatment and adjust therapies accordingly, reducing the risk of over-treatment or side effects and use immune profiling to identify patients who may benefit from targeted immune therapies e.g., IL-6 inhibitors for cytokine storm management (**Paranga, T et al., 2024**).

Biomarkers will continue to play a critical role in the development of new therapeutics. As more is understood about the molecular mechanisms underlying severe COVID-19, novel biomarker-guided therapies targeting specific pathways, such as viral replication, immune dysregulation, or inflammation, will be developed. This could include, small molecule inhibitors targeting key viral proteins , monoclonal antibodies that neutralize SARS-CoV-2 or modulate immune responses ,cytokine-targeting agents to prevent or treat severe inflammation (**Yang, L. et al., 2020**).

6.5 Global Surveillance and Pandemic Preparedness

The emergence of new SARS-CoV-2 variants poses a constant challenge to current diagnostic and therapeutic strategies. Biomarker-based monitoring systems, combined with genomic surveillance, will allow for the rapid detection of variant-specific biomarkers, ensuring that diagnostic tests and vaccines remain effective in the face of evolving strains. This integrated approach will enhance pandemic preparedness by facilitating rapid adaptation of diagnostic tests to detect variants , monitoring the effectiveness of vaccines and therapies against new variants and identifying potential hotspots and predicting future outbreaks (**Aleem, A et al., 2021**).

Biomarkers will play a pivotal role in enhancing global surveillance systems. The use of serological testing to assess population-level immunity and exposure to SARS-CoV-2 will provide valuable data for decision-making and resource allocation. Furthermore, biomarker-based tools for monitoring asymptomatic or pre-symptomatic carriers will help in early containment measures, reducing the spread of the virus (**Oldoni, E . et al., 2021**).

6.6 ***Ethical and Regulatory Considerations***

As new biomarkers are discovered and novel diagnostic technologies are developed, there will be an increased need for regulatory frameworks to ensure the safety, efficacy, and accuracy of these tools. The future will require global cooperation to establish standardized guidelines for biomarker testing, approval processes, and quality control measures to ensure consistency across different laboratories and regions.The widespread use of biomarkers for diagnostic purposes, particularly in large-scale screening programs, will raise concerns related to privacy, informed consent, and equitable access. The development of ethical guidelines and regulatory frameworks for the use of biomarker data will be crucial in addressing these concerns, ensuring that testing programs are conducted transparently and that patient privacy is protected (**Andreoletti, M. et al., 2024**).

7. Conclusion

Biochemical and immunological biomarkers have become essential tools in the fight against COVID-19, facilitating early detection, precise diagnosis, accurate prognosis, and effective management of the disease. Over the course of the pandemic, these biomarkers have demonstrated their immense value in clinical decision-making, allowing healthcare providers to identify infected individuals, assess disease severity, and monitor treatment responses. From viral RNA detection to inflammatory markers and immune system responses, biomarkers provide a comprehensive picture of the infection’s trajectory and the body’s response to SARS-CoV-2.

Despite their promise, the application of biomarkers in COVID-19 management has not been without challenges. Variability in assay sensitivity, the emergence of new viral variants, limited resources in some regions, and the complex biological interplay of biomarkers in individuals with diverse genetic backgrounds and comorbidities all pose significant hurdles. Addressing these challenges requires advancements in technology, greater standardization of biomarker assays, and a stronger global framework for equitable access to diagnostic tools. Additionally, the integration of artificial intelligence and machine learning to interpret biomarker data and improve diagnostic accuracy offers promising solutions to these challenges.

Looking ahead, the future of biomarker use in COVID-19 lies in its integration into precision medicine and personalized treatment approaches. Advances in multi-biomarker panels, real-time monitoring through wearable devices, and the application of AI-driven insights will empower clinicians to make more informed decisions and offer treatments tailored to individual patient profiles. Furthermore, novel biomarker discoveries, combined with technological innovations like point-of-care diagnostics and global surveillance systems, hold the potential to revolutionize how we manage not only COVID-19 but also future pandemics.

Biomarkers are also key to advancing public health strategies, enabling better epidemiological monitoring, faster identification of emerging variants, and more effective vaccine rollouts. By leveraging biomarkers in large-scale surveillance and mass testing programs, public health agencies can better track the spread of the virus, control outbreaks, and prevent future waves.

Ultimately, while challenges remain, the role of biomarkers in managing COVID-19 will continue to grow. By addressing the technical, biological, operational, and ethical issues associated with their use, we can unlock their full potential, improving patient outcomes, accelerating recovery, and strengthening our collective ability to combat global health crises. As research and innovation in biomarker science progress, the tools and strategies developed in response to COVID-19 will likely pave the way for more efficient, accurate, and personalized healthcare in the future.

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