Immunobiochemical aspects in the pathogenesis, diagnosis, and management of novel coronavirus (SARS-CoV-2) infection

Venkata Bharat Kumar Pinnelli[1], Mangala N Sirsikar[2], W Vishnu Vandana[3], Shrabani Mohanty[4], Wilma Delphine Silvia CR[5], Ranjan Kumar Mohapatra[6], Sabitha Vadakedath[7], Venkataramana Kandi[8*]

[1] Professor, Department of Biochemistry,

[2] Associate Professor, Department of Biochemistry

[3] Associate Professor, Department of Microbiology

[4] Professor and Head, Department of Biochemistry

[5]Professor and Head, Department of Biochemistry

[6]Department of Chemistry,

[7]Department of Biochemistry

[8]Associate Professor, Department of Microbiology

[1-4] Vydehi Institute of Medical Sciences and Research Centre, #82, EPIP Area, Nallurhalli, Whitefield, Bangalore-560066 Karnataka

[5]Bowring & Lady Curzon Medical College and Research Institute, Shivajinagar, Bangalore, Karnataka

[6]Government college of Engineering, Keonjhar, Odisha

[7-8]Prathima Institute of Medical Sciences, Karimnagar, Telangana, India

***Author for correspondence:**

Dr. Venkataramana Kandi

Associate Professor, Department of Microbiology

Prathima Institute of Medical Sciences, Karimnagar, Telangana, India

Mobile: 9440704234

Email: ramana20021@gmail.com

ORCID ID

Venkataramana Kandi: https://orcid.org/0000-0002-7197-0448

Abstract

Background: A new coronavirus (SARS-CoV-2) that emerged from Wuhan, Hubei Province, China, has spread throughout the world and is declared a pandemic by the World Health Organization (WHO). A lot remains to be understood of SARS-CoV-2 and the disease (COVID-19). SARS-CoV-2 has until recently been identified as responsible for both asymptomatic and serious life-threatening infections. The unavailability of specific therapeutic agents is a major hurdle in the treatment and management of COVID-19 patients. The present review attempts to evaluate the immunobiochemical aspects of the pathogenesis, diagnosis, and management of SARS-CoV-2 infection.

Main body: This review is a comprehensive evaluation of the data collected through various sources, including Google Scholar, PubMed, and Scopus. The articles were searched and selected using key words such as “Coronavirus disease (COVID-19)”, “Diagnosis of COVID-19”, Pathogenesis of Covid-19”, “management of COVID-19”, “Immunology of COVID-19”, and “Complications of COVID-19”. The study noted that the novel Coronavirus infection could result in an exaggerated immune response, causing a cytokine storm and damaging several organs of the body. The infected patients develop several complications, including immunological, hematological, and biochemical alterations. Consequently, COVID-19 patients may develop cardiovascular, liver, renal, and neurological complications, among others.

Conclusion: An increased understanding of the immunobiochemical aspects of the disease may contribute to better management of SARS-CoV-2-infected persons, as evidenced from the available literature. A holistic approach to the management of COVID-19 patients taking into

consideration the effect of COVID-19 infection on various organs of the body assumes increased significance in patient management.

Key words: Coronavirus, SARS-CoV-2, COVID-19, Immunobiochemical aspects, pathogenesis, diagnosis, management

1. Background

Coronavirus disease 2019 (COVID-19) has become a major health concern worldwide and can be devastating, especially in elderly persons and people with comorbidities. COVID-19 is caused

by SARS-CoV-2, a novel coronavirus. It was found to have increased similarity (~80%) with the SARS-CoV of the 2002-2003 pandemic. SARS-CoV infection also resulted in acute respiratory distress syndrome (ARDS) but with a high mortality rate (up to 10%) [1]. The current pandemic of SARS-CoV-2 was noted to have first emerged from the seafood market in Wuhan, China, signifying its zoonotic link. It was observed that an initial spill-over from animal to human later resulted in human-to-human transmission and subsequent spread throughout the world, causing a pandemic [2].

A group of people with complicated lower respiratory infections/pneumonia presented at various healthcare centers in Wuhan, an industrial hub in Hubei Province of China. The Chinese authorities alerted the observation of the novel viral disease to the World Health Organization (WHO) on December 31, 2019. Because of the uncertainty about the causative agent of the disease, it was initially reported as pneumonia of unknown etiology.

The infection caused by SARS-CoV-2 was initially named Coronavirus disease-19 (COVID-19) by the World Health Organization (WHO). On January 30, 2020, the WHO declared the novel disease as a potential Public Health Emergency of International Concern (PHEIC) as prescribed by the International Health Regulations (IHR, 2005). On February 11, 2020, the WHO declared the novel infectious disease as Coronavirus disease-2019 (COVID-19) and raised an alarm about its contagious nature and potential to cause a pandemic/spread globally. The global spread of the disease was earmarked after the report of a case from the United States of America (USA), the first one to have been reported from outside the Chinese mainland on February 26, 2020. Considering an increase in the number of cases of COVID-19 cases outside China and increasing reports of new cases from various countries of the world, the WHO declared the COVID-19 as a pandemic on March 11, 2020. [3.4]

The mortality rates of infection with COVID-19 varied significantly among the affected countries. A recent study from Italy revealed a mortality rate of 7.2% [5,6]. The case fatality rate, as reported from the Chinese mainland, hovered at approximately 4%. Additionally, the Chinese Center for Disease Control and Prevention (China CDC) reported that only 1% of cases were reported among children under 10 years and adolescents [7]. COVID-19 affects more men than women, as evidenced by the results of a previous study [8]. In the most recent development, a multisystem inflammatory syndrome was reported in children infected with SARS-CoV-2. Children and adults with confirmed COVID-19 were noted to suffer from a clinical condition remarkably similar to Kawasaki disease (a systemic vascular inflammatory condition) and toxic shock syndrome. The patients presented with lymphopenia, hyponatremia, abnormal liver enzymes, thrombocytosis (increased platelets), and elevated activities of inflammatory markers such as erythrocyte sedimentation rate, C-reactive protein, procalcitonin, ferritin, D-dimer, troponin, and interleukin-6 [9]. Additionally, the neurological consequences as a result of SARS-CoV-2 infection are important. As suggested by the WHO, anosmia was recognized as presymptomatic evidence of COVID-19. A recent study evaluated two COVID-19 patients who were diagnosed with inflammatory neuropathy. Histological examination of the olfactory epithelium revealed significant atrophy of the mucosa, leukocytic infiltrates, and axonal damage. Histopathologic examination of the brain confirmed leukocytic infiltration and thrombosis. The study could not confirm whether the inflammation on the neuronal tissue was a direct effect of the infection or otherwise [10]. In view of the COVID-19 disease complexity and due to the unavailability of specific therapeutic drugs, careful management of COVID-19 patients assumes increased significance. Therefore, this review attempts to delineate the importance of

immunobiochemical aspects of SARS-CoV-2 infection and their potential role in COVID-19 disease management.

Main text

2. Structure of Coronaviruses

Coronaviruses belong to the *Coronaviridae* family under the Nidovirales group. They have enveloped viruses with a large positive-sense, single-stranded ribonucleic acid (RNA) genome measuring ~30 kb. They have a wide host adaptability that includes humans, birds, rats, mice, chickens, cattle, turkeys, pigs, cats, dogs, rabbits, wild ruminants, beluga whales, and horses. In animals, coronaviruses generally cause epidemic outbreaks of respiratory and intestinal disease. According to molecular and phylogenetic studies, coronaviruses are classified into four genera: α, β, γ, and δ coronaviruses. The α and β coronaviruses are associated with infections in mammals, including humans, and the γ and δ coronaviruses cause infections in birds [11.12].

Coronaviruses possess four structural proteins that include the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins [13]. The spike proteins extend outside the viral particle and are made up of transmembrane trimetric glycoproteins. The spike proteins make the virus look like a crown (in Latin corona means garland or crown; in Greek korṓnē means garland), from which the name Coronavirus is derived. They function as viral determinants and help the virus attach to host cells. A spike protein has two functional subunits; the S1 subunit has a receptor-binding domain (RBD), which is responsible for the binding of the virus to the host cell receptor. The S2 subunit has two heptads, the repeat regions HR1 and HR2, and it facilitates the adhesion of the viral particle to the host cell membrane. A previous study revealed that the angiotensin-converting enzyme 2 (ACE2) receptors present on various cells facilitate viral

attachment in the case of SARS-CoV [14]. Recent studies have demonstrated that the spike proteins of the novel SARS-CoV-2 also bind to ACE2 receptors, triggering the infection process [15-17]. The expression of ACE2 receptors has been found increasingly on type II alveolar epithelial cells of the lungs, heart, ileum, kidney, endothelium, and bladder [18, 19, 20].

In general, the life cycle of a virus within the host consists of the various phases that include attachment, penetration, biosynthesis, maturation, and release. After the virus binds to the host cell (attachment), it penetrates the cell through endocytosis or membrane fusion. Later, the viral nucleic acid is released inside the host cell. The viral nucleic acid replicates along with the host cell nucleic acid, after which the viral mRNA is used to produce viral proteins (biosynthesis) and develop new viral particles, which undergo maturation and are released. After the virus enters the cell, it becomes exposed to the endosomal proteases present in the host cells [21]. Within the endosome, the fusion peptide is exposed after the S1 subunit is cleaved and inserted into the host cell membrane. The S2 region undergoes a conformational change to bring the HR1 and HR2 regions together. This leads to membrane fusion and release of the viral nucleic acid into the host cytoplasm followed by replication, which results in the formation of daughter viral particles. The viral particles are released when the host cell disintegrates and the virus spreads to the other cells [22]. In addition to the S protein, the plasma membrane-associated type II transmembrane serine protease (TMPRSS2) was found to facilitate the entry of SARS-CoV-2 into the host cell [23]. Previous research has suggested that the RBD of SARS-CoV-2 binds to ACE2 with a higher affinity than SARS-CoV [24].

3. Immune sensing

Viral antigens are recognized by cells of the innate immune system, such as antigen-presenting cells (APCs). These cells process and present antigens to cells of the cell-mediated immune

system, such as natural killer cells (NK) and TCD8+ cytotoxic cells. Hence, both innate and adaptive immune systems are activated. This results in the enormous stimulation and secretion of pro-inflammatory mediators that include cytokines and chemokines. Such an excessive stimulation of inflammatory cytokines creates a storm that results in damage to the tissues, organs, and vascular endothelium, causing multi-organ failure and even death [25, 26].

The innate immune response is the first line of defense, wherein cells such as phagocytes, monocytes, and others possess pattern recognition receptors (PRRs) that play a key role in the identification of pathogen-associated molecular patterns (PAMPs). PAMPs include viral nucleic acids (RNA/DNA), damage/danger-associated molecular patterns (DAMPs) such as adenosine triphosphate (ATP), apoptosis-associated speck-like protein with caspase-recruitment domain (ASC) oligomers expressed on virus leading to secretion of interleukin-6 (IL-6), interferon-γ (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), and interferon γ-induced protein (IP-10). These pro-inflammatory cytokines were noted to be released into the blood of affected patients [27, 28].

PRRs such as endosomal Toll-like receptors (TLR-3 and TLR-7) recognize PAMPs in the extracellular milieu, the activation of which leads to the nuclear translocation of transcription factors such as the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and interferon-regulatory factor family 3 (IRF3). Triggering NF-kB causes an increase in the expression of IL 1, IL 6, and tumor necrosis factor-α (TNF-α). The second set of cytosolic PRRs, such as retinoic acid-inducible gene I (RIG-I) like receptors (RLRs) such as RIG-I, melanoma differentiation--associated protein-5 (MDA-5), and laboratory of genetics and physiology-2 (LGP-2), recognize intracellular PAMPs. RIG-1 and MDA5 activation results in the stimulation of IRF3 through the mitochondrial adaptor antiviral signal (MAVS) protein. In turn, these trigger

increases in the expression of type 1 IFN, which is considered most important for antiviral defense. This leads to the activation of the IFN-α receptor complex (IFNAR) and causes phosphorylation/activation of signal transducers and activators of transcription (STAT) family, transcription factors 1 and 2 [29-32]. Additionally, the binding of DAMPs with RLRs triggers the synthesis of inflammasomes, which trigger the conversion/maturation of procaspase-1 to caspase-1 and pro IL-1β to IL-1β, which are potent pro-inflammatory cytokines [33].

Because of the activation and production of proinflammatory cytokines by the innate immune system, the host defense mechanism then channelizes the adaptive immune system against infection. Such a response, along with the activities of T lymphocytes, plays a key role in defense against viral infection of cells. The activation of TCD4+ and TCD8+ cells induces the activation of B- cells (antibodies), cytotoxic cells, and other immune cells, which are directed against viral entry and replication [34].

Most virus-infected cells are destroyed by perforin and granulysin proteins secreted by natural killer (NK) cells and TCD8+ cells. APCs and cytotoxic T cells undergo apoptosis to minimize damage to host cells. Genetic factors and other acquired causes may compromise the ability of NK and cytolytic T cells to destroy surplus APCs. This causes unwarranted and extended stimulation of both innate and adaptive immunity. Such a phenomenon is observed in patients infected with COVID-19, wherein there is an increased accumulation of pro-inflammatory cytokines such as TNF, interferon-γ, IL-1, IL-6, IL-18, IL-33, and others such as activated macrophages causing macrophage activation syndrome (MAS), resulting in a cytokine storm, as shown in **Figure 1**.

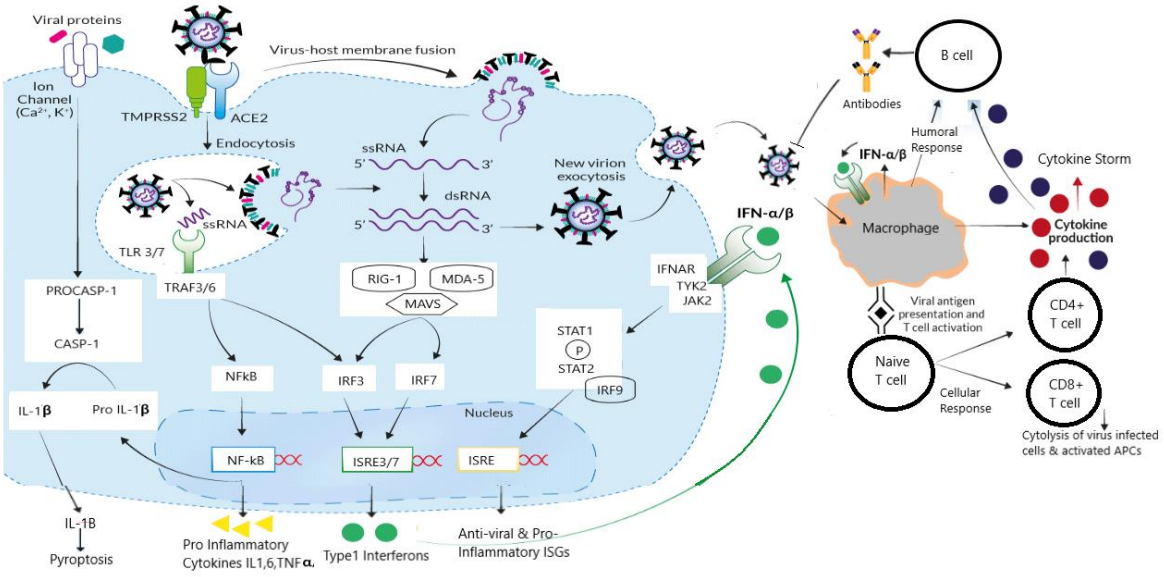

Figure 1: Image depicting the process resulting in cytokine storm as a result of SARS-CoV-2 infection

Therefore, COVID-19 patients are predisposed to acute respiratory distress syndrome (ARDS) and multiorgan failure [35, 36].

4. Cytokine storm

The cytokine storm is caused by an increased secretion of pro-inflammatory cytokines from activated macrophages and monocytes. Some factors that are elucidated for cytokine storm include impaired viral clearance due to immune evasion by the virus, presence of low levels of type 1 INF (T_1:INF) due to inhibition of T_1:INF signaling as it happens in antibody directed enhancement (ADE), deficiency or inactivation of MDA5, increased neutrophil extracellular traps (NETs), and pyroptosis, which is an inflammatory and caspase 1-dependent programmed cell death. All these factors lead to the release of pro-inflammatory intracellular contents [37].

5. Immune evasion

Viruses may escape host immune responses by evading PRR sensing. They can do so either by evading or antagonizing PRR action. SARS-CoVs and most likely SARS-CoV-2 could affect the inactivation of cytoplasmic RNA sensors (RIG-I and MDA5) [34]. ORF9b suppresses MAVS signaling, which is required to induce the nuclear translocation of interferon regulatory factor 3 (IRF3) [37]. IFN release is suppressed by counteracting T1IFN signaling and the transcription factor phosphorylation of STAT family proteins [38]. Additionally, TNF receptor-associated factors (TRAFs) 3 and 6 are blocked, which are important for the activation of IRF3 and IRF7 [34]. It was noted that the novel coronaviruses replicate in the host cells by suppressing the host innate immune system and antiviral response mechanisms. Furthermore, apart from stopping IFN signaling, the virus activates alternative inflammatory pathways by secreting accessory viral proteins such as open reading frame 3a (ORF3a), ORF8b, and E proteins, which improve the formation of inflammasomes and the release of IL-1b and IL- 18 [39, 40]. Viral non-structural proteins (NSP9 and NSP10) have been shown to suppress the NKRF gene, which codes for endogenous NF-kB repressor proteins [41, 42]. These factors could contribute to enhanced inflammation and cytokine storm.

6. Transmission pattern

According to the latest guidelines and from observations made by the Chinese and several other scientists throughout the world, the novel CoV SARS-CoV-2 appears to transmit mainly through droplets, aerosol inhalation, and mucosal contact transmission. Droplets and aerosols are viral particles containing respiratory and oral secretions that are released into the environment when an infected person coughs, sneezes, and talks. Aerosols are also produced during various laboratory (centrifuging) and clinical procedures (tracheal intubation). The distance travelled by

the droplets depends on the size of the droplets, wherein droplets >5 μm may not travel more than one meter, but those that measure <5 μm may be floating/suspended in the air for longer periods and may potentially be carried to a longer distance. Transmission by contact (direct or indirect) may occur when a person comes into skin contact with a surface, object, or a fomite contaminated with the virus, which accidentally enters through mucosal contact with mouth, nose, and conjunctiva. [43, 44].

7. Clinical manifestation and diagnosis

Patients infected with SARS-CoV-2 develop fever, cough, and dyspnea, with pneumonia occurring in complicated cases. It was also observed that several infected patients remain asymptomatic or develop only mild symptoms. The diagnosis of COVID-19 is made based on the travel history of a person (recent travel to places where the disease has shown community spread, contact with an infected or suspected COVID-19 patient), comprehensive clinical, and laboratory examination. The diagnostic methods used include computed tomography (CT) scans of the lungs, antigen/antibody detection by enzyme-linked immunosorbent assay (ELISA), and confirmation by nucleic acid amplification tests such as polymerase chain reaction (PCR) [45].

7.1.Molecular diagnostic methods

Whole genome sequencing (WGS) and real-time reverse transcriptase polymerase chain reaction (rt-RT-PCR) are two frequently applied molecular diagnostic methods for SARS-CoV-2 infection. In view of the high cost associated with WGS, rt-RT-PCR is the most common, effective, and preferred tool for diagnosing COVID-19 from specimens that include nasal and nasopharyngeal swabs, sputum, and other respiratory secretions. Currently, rt-RT-PCR is recommended as the gold standard method to diagnose SARS-CoV-2 infection [45].

7.1.2. Patient Screening

The World Health Organization (WHO) recommended procedure for suspecting and diagnosing the COVID-19 caused by the SARS-CoV-2 is depicted in **Figure 2** [44].

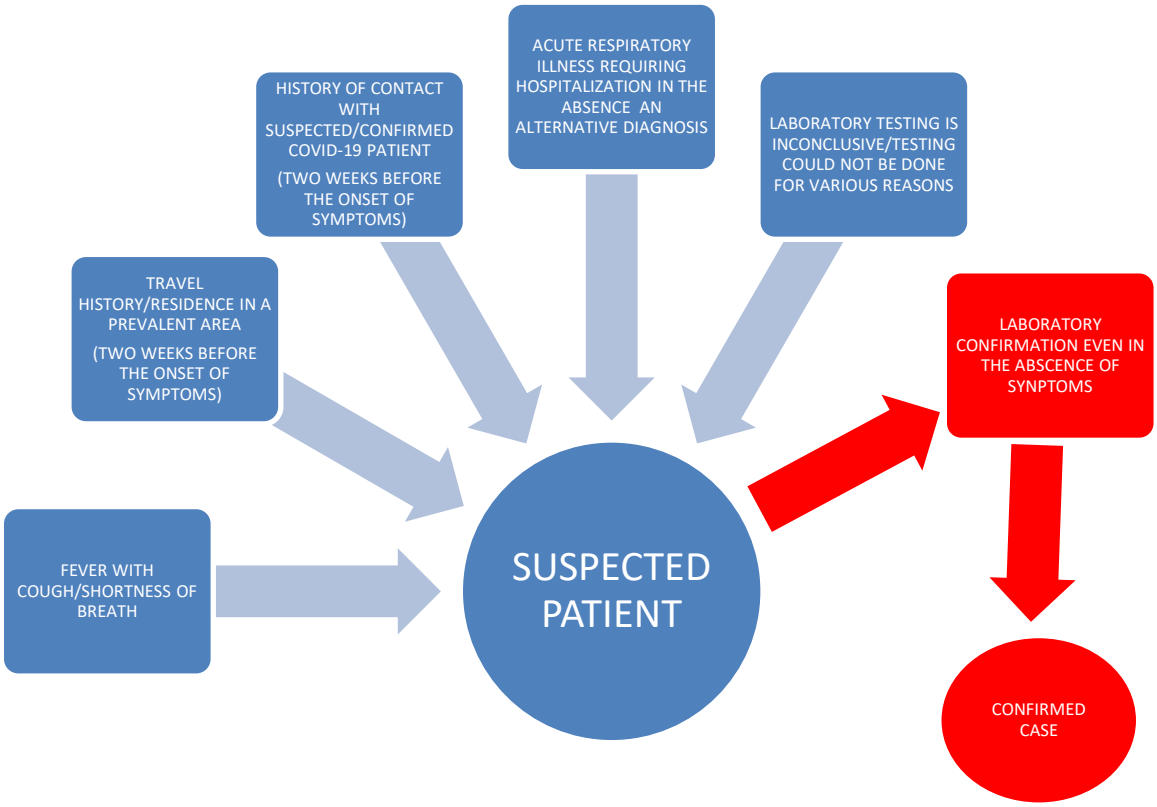

Figure 2: Diagrammatic representation of identifying a suspected person and diagnosing COVID-19

All patients who present with acute respiratory illness, along with fever and cough, dyspnea, and a recent history of travel to a place that has reported community transmission of COVID-19 two weeks before the onset of symptoms should be considered suspected patients. Additionally, patients who present with an acute respiratory illness and give a history of coming into close proximity with a COVID-19-positive or suspected COVID-19 case two weeks before the onset

of symptoms are considered suspected patients. All patients with acute and complicated respiratory illness, presenting with symptoms and requiring hospitalization with no alternative diagnosis were defined as suspected patients. All persons without conclusive evidence of COVID-19 **and** persons who could not be tested for various reasons are considered suspected COVID-19 patients [43]. All other persons who are tested and return positive for SARS-CoV-2 infection are labelled as confirmed cases, irrespective of clinical signs and symptoms [44].

8. Biochemical Monitoring in COVID-19 Patients

During the current COVID-19 pandemic, clinicians and clinical laboratories are engaged in suspecting the cases and performing clinical and laboratory diagnoses. Additionally, it is important to manage infected patients using various biochemical, hematological, and inflammatory markers. Estimation of these parameters allows physicians treating COVID-19 patients to assess disease severity and prognosis, as shown in **Figure 3** [46, 47].

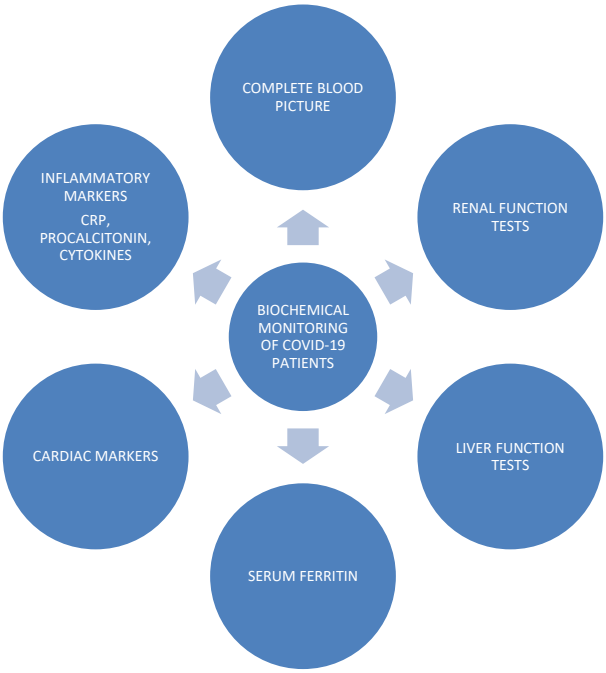

Figure 3: Diagrammatic representation of the biochemical monitoring of COVID-19 patients

Complete blood picture

The routine blood indicators included white blood cell count (WBC), lymphocyte count and ratio, neutrophil count and ratio, monocyte count and ratio, basophil count and ratio, eosinophil count and ratio, platelet count (PLT), PLT-large cell ratio, PLT volume distribution width (PDW), red blood cell (RBC) count, RBC volume distribution width-CV, RBC volume distribution width-SD, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular-hemoglobin concentration (MCHC), mean platelet volume (MPV), hemoglobin (HB), immature granulocyte counts and ratio.

Biochemical markers such as albumin, globulin, albumin to globulin ratio, blood gas, serum calcium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), AST/ALT, creatine kinase (CK), CK isoenzyme MB (CK-MB), sodium, potassium, chloride, magnesium, urea, uric acid, creatinine, lactate dehydrogenase (LDH), inorganic phosphorus, anion gap, direct bilirubin, total bilirubin, total bile acids, total protein, D-dimer, C-reactive protein (CRP), and procalcitonin (PCT) are estimated during the management of COVID-19 patients.

8.1.Lymphopenia

The cause of lymphopenia in COVID-19 patients may be due to the infection of lymphocytes by the virus resulting in their death. Lymphocytes express ACE2 receptors and may become a direct target of Coronavirus infection. An acute decline in lymphocyte counts might be related to the loss of lymphocyte function and to the direct damage caused by the novel Coronavirus virus

virus to organs such as the thymus and spleen. Additionally, inflammatory cytokines may cause lymphocyte apoptosis.

Previous research has confirmed that tumor necrosis factor (TNF-α), interleukin (IL)-6, and other proinflammatory cytokines can damage lymphocytes. The inhibition of lymphocytes may also be caused by metabolic disorders such as hyperlacticacidemia. Lymphopenia could be an effective and reliable indicator of the severity of the infection and the need for hospitalization among COVID-19 patients [47].

8.2.Neutrophil lymphocyte ratio

The ratio of neutrophils to lymphocytes (NLR) is a value calculated by using the absolute neutrophil and lymphocyte counts present in the blood. It is used to predict potential systemic inflammatory conditions. An increase in the counts of neutrophils and a decrease in the lymphocyte counts predict the severity of inflammation and the extent of damage to the immune system, respectively. The NLR can be quickly calculated by routine blood tests, which help in identifying SARS-CoV-2 infection at an early stage. Increased NLR indicates severity and is an independent risk factor for in-hospital mortality. The NLR, platelet-lymphocyte ratio, and monocyte-lymphocyte ratio, when considered in combination, can be used to predict the severity of COVID-19 [48, 49].

8.3.Role of ferritin and hyperferritinemia syndrome in COVID -19

Ferritin is a storage protein that participates in iron metabolism. By structure, ferritin contains L and H subunits, which are expressed in the lung and heart, respectively. The H subunit is involved in the inflammatory mechanism by participating in myeloid and lymphoid cell proliferation and stimulating TIM-2, a specific ferritin receptor. H-ferritin plays a key role in

immunomodulatory and pro-inflammatory activities by activating several inflammatory mediators, such as IL-1β [48].

Ferritin was found only in the lymph node B area, suggesting its role as an antigen that stimulates macrophage activation related to hyperferritinemia [50]. Previous research has suggested that the activities of serum ferritin correlate with the intensity of systemic and pulmonary inflammation, confirming the role of hyperferritinemia in assessing the severity of disease among COVID-19 patients. Possible mechanisms supporting such a consequence include increased ferritin synthesis by proinflammatory cytokines such as IL-lβ, TNF-α, and IL-6. This results in increased inflammation that causes cell damage and release of ferritin. It was also noted that during metabolic disorders such as acidosis, the microvascular environment promotes the accumulation of reactive oxygen species (ROS), which in turn facilitates the uncoupling of iron from ferritin. Free iron participates in Haber-Weiss and Fenton reactions, which produce reactive species such as hydroxyl radicals, resulting in serious injury to the cells and promoting inflammation [51].

8.4.Role of cardiovascular markers in patients with COVID-19

The activities of LDH, CK, CK-MB, myoglobin (Mb), cardiac troponin I (cTnI), alpha-hydroxybutyrate dehydrogenase (α-HBDH), AST, and the N-terminus of the prohormone brain natriuretic peptide (NT-proBNP) were elevated in varied concentrations in COVID-19 patients [52, 53]. An elevation in laboratory cardiac markers could be used as a prognostic tool in predicting the necessity of intensive care and critical management of the patients along with the disease outcome [54].

The available literature supports the assumptions that SARS-Co-V-2 infection predisposes COVID-19 patients to various cardiovascular complications, including heart failure, myocardial infarction, cardiac arrhythmias, and myocarditis.

The proposed mechanisms underlying myocardial injury and elevated troponin include binding of the virus to ACE2 present on myocytes, which can activate the renin-angiotensin system and alter ACE2 signaling pathways, thereby causing acute myocardial injury. Additionally, complicated COVID-19 infections present with fulminant inflammation/cytokine storms that damage various organs of the body, causing multi-organ failure. Myocardial injury may be caused by increased inflammatory and hypoxic conditions as a consequence of acute respiratory illness. Because of the elevated activities of cytokines, causing systemic inflammation, there is an increase in the prothrombotic milieu, which generates shear stress in the coronary blood vessels, causing increased blood flow. This results in the rupture of plaque, causing vascular blocks and myocardial infarction. Anti-microbial agents used in treating and managing patients infected with COVID-19 may also contribute to cardiovascular events, as evidenced from the available literature. Since SARS-CoV-2 interacts with the renin-angiotensin system, which is a significant factor in balancing body electrolytes, and because of the excessive inflammatory environment, studies have reported hypokalemia and suggested that COVID-19 patients may develop electrolyte imbalance and cardiovascular events such as tachyarrhythmias [55].

8.5.The clinical course of COVID-19 patients as observed in relation to the activities of cardiac troponin

Mild – Patients hospitalized with COVID-19 have been noted to show elevated/fluctuating activities of troponin, typically remaining well below the 99th percentile upper reference limit. This appears to be the most common pattern of troponin elevation in patients with COVID-19

and is often associated with no cardiac symptoms. This pattern has been described in patients with COVID-19 who survived after hospitalization.

Moderate time-limited – Elevated activities of troponin, higher than the 99th percentile of the upper reference limit, which stabilized to normal on subsequent days, were observed in patients who were clinically suspected of developing myocarditis or stress cardiomyopathy.

Progressive – Some patients with moderate troponin elevation at hospital admission suffer clinical deterioration with respiratory failure accompanied by progressive troponin elevation, along with elevations in the activities of D-dimer, IL-6, ferritin, and LDH. An accelerated rise in the activities of troponin after the second week of hospitalization was observed with progression to cytokine storms among non-survivors [56]

8.6.Role of CRP and procalcitonin in COVID-19

CRP is an inflammatory substance that is secreted during microbial infections and inflammatory conditions. It is an acute-phase protein secreted by IL-6 in the liver. During injury, infection, and inflammation, there is an increase (>0.6 mg/dL) in the activities of CRP, which returns to normal (<0.6 mg/dL). Therefore, it is considered a potential marker for disease prognosis. As evidenced by the available literature, it was observed that the activities of CRP were significantly increased in COVID-19 patients who required ventilation.

Procalcitonin (PCT) is a protein/peptide that is secreted by various cells of the human body in response to microbial infections. Research thus far indicates that the activities of PCT either do not increase or are only moderately elevated during viral infections [57]. The higher PCT activities among severe COVID-19 patients indicate concomitant bacterial or secondary microbial infections.

Activities of PCT could be used as an early indicator in assessing the risk of death due to sepsis in hospitalized patients. Serum activities of PCT <0.5 µg/L could rule out secondary microbial infections. Adverse clinical outcomes can be predicted when the activities of PCT are ≥0.5 µg/L. A progressive increase in PCT activities may suggest the severity of bacterial infection/sepsis and indicate poor prognosis and risk of death [57, 58].

8.7.Role of D-dimer in COVID-19

SARS-CoV-2 binds to ACE2 receptors on type II pneumocytes and possibly on vascular endothelial cells and causes lysis of the cells. This leads to direct activation of the endothelium, causing procoagulant activity. It then results in the accumulation of fibrin deposits in pulmonary microcapillary venous vessels. Fibrin deposits cause a compensatory mechanism of increased plasminogen at the beginning, but as the disease progresses, fibrin deposits fail to break down, as evidenced by increased d-dimer activity [59, 60, 61].

8.8.Role of LDH in COVID-19

LDH is an intracellular enzyme that is essential for the conversion of cell sugars (lactate, pyruvate) into energy. LDH is present in the cells of various organs of the body, including the liver, lung, heart, kidneys, and others. LDH is found in five isoenzyme types, including LDH1-LDH-5. Each LDH isoenzyme is associated with a particular organ of the body. LDH isoenzymes 1 and 2 are found in RBCs and heart tissue. LDH isoenzymes 4 and 5 are present in the liver and skeletal muscles. LDH isoenzyme 3 is found in the lungs, lymphoid tissues, severe infections, tissue injury, and inflammation, causing cell lysis and LDH release. Because LDH isozyme 3 is present in lung cells, infection with SARS-CoV-2 may cause damage to the lung

tissue and release of LDH 3, which can be analyzed among COVID-19 patients to assess disease severity [59]. Increased activities of different LDH isoenzymes may predict multiple organ failure and influence the clinical outcome, as evidenced from the correlation of increased LDH with poor clinical outcomes in severe COVID-19 patients [59].

8.9.Role of serum amyloid A

Serum amyloid A (SAA) is an acute phase protein secreted primarily by liver cells. Patients with severe acute respiratory syndrome had significantly increased activities of SAA. This suggests the role of SAA as a biomarker in monitoring the progression of acute respiratory diseases. Patients with respiratory viral infections usually have clinical symptoms after 36-48 hours of infection. The activities of SAA gradually increased and reached a peak on the fourth day of infection. During recovery, the activities of SAA continuously decrease at a rate faster than that of CRP. The serum activities of SAA can indicate/identify the severity of infection in COVID-19 patients [62]. In normal subjects, the serum activities of SAA remain <10 mg/L, and severely ill patients present with SAA >10 mg/L, signifying the importance of SAA as an indicator of disease severity among COVID-19.

8.10. Role of renal function test in COVID-19

The kidneys may be affected in COVID-19 patients, who may develop acute kidney injury (AKI) and present to the hospital with proteinuria. Because of the increased expression of ACE-2 receptors in glomerular cells, SARS-CoV-2 infects renal tubular epithelial cells and cells in Bowman's capsule (podocytes). This may cause mitochondrial dysfunction, acute tubular necrosis, the formation of protein reabsorption vacuoles, collapsing glomerulopathy, and protein leakage in Bowman's capsule [63, 64].

The activities of serum urea and creatinine could be used to distinguish severe COVID-19 from mild cases, as they reflect glomerular filtration rates/kidney function. These biochemical parameters may be considered early indicators and used to manage patients with severe COVID-19.

9. COVID-19 therapeutics

Similar to SARS-CoV and MERS-CoV, there is currently no clinically proven specific antiviral agent available against SARS-CoV-2 infection. The supportive treatment, including oxygen therapy, conservative fluid management, and the use of broad-spectrum antibiotics to cover secondary microbial infections are some of the important management strategies. According to research on the molecular mechanisms of Coronavirus infection and the genomic organization of SARS-CoV-2, there are several potential therapeutic targets to repurpose existing antiviral agents or develop effective interventions against this novel coronavirus [6].

The therapeutic efficacy of GS-3754 (remdesivir), an adenosine nucleoside analog, which is currently under trial, has been found to be useful in the treatment of the Ebola virus. Research is currently underway to find its potential usefulness in the treatment of SARS-CoV, MERS-CoV, and other viruses that can pose a serious public health threat against whom there is no specific therapeutic drug [65].

The utility of lopinavir/ritonavir and ribavirin, which have shown promise against SARS-CoV, and MERS-CoV, in treating COVID-19 patients remains to be adequately researched [66]. A combination of lopinavir/ritonavir and interferon-beta-1b, ribavirin, and interferon, which showed promise against MERS-Co-V, also remains to be tested for their efficacy against SARS-CoV-2 infection [67].

HIV protease inhibitors such as lopinavir/ritonavir (LPV/r) in combination and nelfinavir were recommended for the treatment of COVID-19 pneumonia [68, 69, 70]. Baricitinib is another potential drug for COVID-19 treatment that inhibits viral entry into cells and inflammation [69]. Other drugs recommended include Remdesivir, chloroquine, arbidol, hydroxychloroquine, ivermectin, and sitagliptin, which could demonstrate effective antiviral activity against SARS-CoV-2 infection [70, 71, 72, 73, 74, 75].

Recently, the anti-rheumatic drug tocilizumab, with its anti-inflammatory properties, was noted to be effective in treating severe cases of COVID-19 [76]. Even teicoplanin, an antibacterial antibiotic that was found to inhibit MERS-CoV, was suggested to treat SARS-CoV-2 infection [77].

First, a study from Spain reported a series of COVID-19 cases in which patients who were on mechanical ventilation were treated with allogenic adipose tissue-derived mesenchymal stromal cells (AT-MSCs). The study observed that cases treated with AT-MSCs showed clinical improvement [78].

COVID-19 patients suffer from systemic inflammation and extensive endothelial vasculitis, which predisposes them to coagulopathy. This may result in embolic stroke and other cardiovascular events, such as myocardial infarction, ischemia, and renal failure. A recent study reported the efficacy of the antihypertensive drug iloprost in the treatment of such cases [79]

Extensive research involving small molecule compounds, interfering mRNAs such as microRNAs, small interfering RNAs (siRNAs), RNA aptamers, ribozymes, antisense oligonucleotides, antiviral cytokines such as IFNs, and therapeutic antibodies to establish their

efficacy against SARS-CoV, MERS-CoV, Ebolavirus, influenza virus, human immunodeficiency virus (HIV), and other enveloped viruses is underway [80].

Extensive research is underway, which is currently in different phases of clinical trials, to identify novel therapeutic drugs/vaccines to treat, control, and prevent COVID-19 [81, 82, 83, 84, 85, 86, 87].

10. Conclusion

The novel Coronavirus disease has now been in existence for more than seven months. The infection is spreading at an increased pace, and we have no clue when the rates of infection become stabilized. COVID-19 is a complex disease that affects all age groups, causing infections ranging from asymptomatic to severely debilitating and deadly consequences. The present literature also indicates the immunomodulatory abilities of the novel Coronavirus, which results in a cytokine storm. An increased understanding of the immunobiochemical aspects of the disease may contribute to better management of SARS-CoV-2-infected persons. Additionally, because there is neither a specific therapeutic drug nor an approved vaccine against SARS-CoV-2 infection, it poses a challenge to clinicians treating COVID-19 patients. A holistic approach to the management of COVID-19 patients assumes increased significance, both in controlling the spread of infection and minimizing morbidity and mortality.

Abbreviations

COVID-19: Coronavirus disease-19

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus-2

SARS-CoV: Severe Acute Respiratory Syndrome Coronavirus

ACE2: Angiotensin-converting enzyme 2

RBD: Receptor-binding domain

TMPRSS2: Plasma membrane-associated type II transmembrane serine protease

APCs: Antigen-presenting cells

NK: Natural killer cells

PRRs: Pattern recognition receptors

PAMPs: Pathogen-associated molecular patterns

DAMPs: Damage/danger-associated molecular patterns

ATP: Adenosine triphosphate

IL-6: Interleukin -6

IFN-γ: Interferon-γ

IP-10: Interferon γ-induced protein

TLR: Toll-like receptors

IRF3: Interferon-regulatory factor family 3

TNF-α: Tumor necrosis factor-α

RIG-I: Retinoic acid-inducible gene I

RLRs: Retinoic acid-inducible gene I-like receptors

MDA-5: Melanoma differentiation--associated protein-5

LGP-2: Laboratory of genetics and physiology-2

MAVS: Mitochondrial adaptor antiviral signal

IFNAR: IFN-α receptor complex

STAT: Signal transducers and activators of transcription

MAS: Macrophage activation syndrome

ARDS: Acute respiratory distress syndrome

NETs: Neutrophil extracellular traps

TRAF: TNF receptor-associated factors

ORF3a: Open reading frame 3a

NSP9: Nonstructural proteins

ELISA: Enzyme-linked immunosorbent assay

WGS: Whole genome sequencing

rt-RT-PCR: Real-time reverse transcriptase polymerase chain reaction

cTnI: Cardiac troponin I

α-HBDH: alpha-hydroxybutyrate dehydrogenase

NT-proBNP: N-terminal of the prohormone brain natriuretic peptide

LDH: Lactate dehydrogenase

CRP: C-reactive protein

SAA: Serum Amyloid A

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