Chapter 7
COVID Diagnostics: From Molecules to Omics

Chemedzai Chikomba, Siphelele Dlamini, Jaya A. George, and Taryn Pillay

Abstract The identification and genetic sequencing of a novel coronavirus was key to the diagnosis and management of the global pandemic. An understanding of the SARS-CoV-2 structure and mechanism of injury is vital to explaining the disease course and the pathophysiology of the signs and symptoms observed. This particularly as the presentation, disease course, and severity are noted to be highly variable. The role of the spike protein and angiotensin-converting enzyme 2 (ACE-2) receptor in immune response and viral entry provides great insight into current and future diagnostics and therapeutics. This article reviews the traditional diagnostic methods, which include molecular testing methods, antigen testing, and antibody testing. The gold standard for diagnosis of COVID-19 is reverse transcriptase polymerase chain reaction (RT-PCR). There have been multiple improvements to these principles to help optimize the sensitivity, specificity, and user friendliness of the method. In addition, advancements in gene sequencing and identification have been integral to identifying variants and managing outbreaks. Serological and immunological testing have made significant contributions to the management of the COVID-19 pandemic, each with its unique benefits and limitations. A growing role of the laboratory is in triaging patients to determine which patients will most benefit from hospitalization and specialized care. This is imperative for rationalizing resources during outbreaks. As we learn to live with the pandemic, novel testing methods include the use of multiomic technologies and the greater utility of point of care.

Chemedzai Chikomba and Siphelele Dlamini contributed equally with all other contributors.

C. Chikomba · S. Dlamini · T. Pillay
Department of Chemical Pathology. National Health Laboratory Services and University of Witwatersrand, Johannesburg, South Africa

J. A. George (✉)
Wits Diagnostic and Innovation Hub, University of Witwatersrand, Johannesburg, South Africa
e-mail: jaya.george@wits.ac.za

P. C. Guest (ed.), *Application of Omic Techniques to Identify New Biomarkers and Drug Targets for COVID-19*, Advances in Experimental Medicine and Biology 1412, https://doi.org/10.1007/978-3-031-28012-2_7

Keywords COVID-19 · RT-PCR · COVID-19 antibodies · COVID-19 antigen tests · IL-6 assays for COVID-19 · Omics

1 Introduction

In December 2019, Chinese health authorities identified an outbreak of pneumonia of unknown origin with high mortality, which raised intense concern not only in China but also internationally as well. In attempts to control the spread of the disease, Chinese authorities isolated infected people and monitored close contacts. They characterized the clinical presentation and sought to develop diagnostic and treatment modalities. By January 2020, they isolated a novel coronavirus, and the genetic sequencing of this virus [1] enabled the development of molecular tests specific for the virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The disease spreads rapidly, and the World Health Organization (WHO) declared it a global pandemic in March of 2020, with more than 100,000 cases and 4000 deaths reported worldwide at that time [2]. In Africa, the first case was reported in February 2020, and by March of the same year, cases were reported from across the continent. To date, over 650 million cases have been reported globally, with over 6 million deaths.

2 Transmission and Pathogenesis

SARS-CoV-2 belongs to a group of viruses of the *Betacoronavirus* genus, which includes SARS Co-V and Middle East respiratory syndrome coronavirus (MERS-CoV). SARS-CoV-2 shares 75–80% of its viral genome with SARS-CoV [3]. It is an enveloped, single-stranded RNA virus and has four structural proteins: the spike, nucleocapsid, membrane, and envelope proteins. These proteins play a key role in the pathogenesis of disease. The spike protein is used for viral entry via the angiotensin-converting enzyme 2 (ACE2) receptor and causes membrane fusion which is important for viral entry into cells [4–6]. The spike protein is also the primary target of neutralizing antibodies and the focus of vaccine development.

The major route of transmission is from infected patients via respiratory droplets and possibly contact with fomites and when aerosols are generated during medical procedures like endotracheal intubation [7, 8]. Transmission can occur in asymptomatic people and during the early incubation phase [9]. Viral load in the upper respiratory tract appears to peak around the time of symptom development, with viral shedding starting 2–3 days before the onset of symptoms [10]. Presymptomatic transmission is thought to be a major route for the spread of infection, with

modeling studies estimating transmission rates ranging from 48% to 62% [11]. While perinatal transmission from mother to babies can occur, this is rare [12].

Disease presentation is varied, with many patients remaining asymptomatic or having mild disease and quick recovery. The most common symptoms are flu-like with a sore throat, fever, cough, muscle pains, and headache [13]. In severe cases, patients may go on to develop a pneumonia and then acute respiratory distress syndrome (ARDS) [14].

As severe COVID-19 presents with multisystem involvement, the role of the laboratory is key in not only the diagnosis of the disease but also in detecting system involvement and in monitoring the disease [15–20] (Table 7.1). Reported cases of COVID-19 infection and death appear to be far less in Africa compared to the rest of the world [21]. This may be due to a number of factors such as the relatively young population and perhaps unexplored protective genetic factors [22]. It may also be a result of underreporting as testing capacity is less in South Africa than many other areas in the world. Large swathes of the population live in rural areas where communities may have limited access to healthcare facilities. Risk factors for COVID-19 include older age, obesity, diabetes, hypertension, and existing kidney disease. There is some evidence that human immunodeficiency virus (HIV) and tuberculosis, both of which are major causes of death in sub-Saharan Africa, increase risk for morbidity and mortality from COVID [23, 24]. With limited healthcare resources, it is important to look at rapid diagnostic tests for COVID-19 as well as for complications of the disease. This review highlights traditional diagnostic and point of care tests for COVID-19 and related diagnostics, as well as the potential role of "Omics" in the laboratory management of this disease.

3 COVID-19 Diagnosis

3.1 Traditional Diagnostic Methods

SARS-Co-V-2 diagnosis is based on the clinical suspicion, laboratory investigations, and imaging modalities. Laboratory testing for the diagnosis of SARS-CoV2 is based on the identification of viral nucleic acid, antigen, or host-antibody responses. Table 7.2 summarizes these tests and their clinical utility.

Molecular tests allow for viral RNA detection by using nucleic acid amplification and detection techniques [25]. Among these and widely used globally is real-time reverse transcriptase polymerase chain reaction (RT-PCR). The Wuhan scientists isolated the virus from a bronchoalveolar lavage specimens and used a combination of molecular techniques including Sanger, Illumina, and nanopore sequencing to establish the complete genome.

The principle of molecular testing is that different genome regions are used to develop primers and probes for the PCR tests. Targeted regions of the viral genome include the RNA polymerase region, spike, nucleocapsid, and envelope proteins

Table 7.1 System involvement and their laboratory tests

System involved	Severe disease presentation	Pathophysiology	Diagnostic test
Pulmonary	Severe hypoxemia Acute respiratory distress syndrome (ARDS) Respiratory failure and death (if untreated)	Endothelial barrier disruption and impaired oxygen diffusion capacity are characteristic features of COVID-19 in the respiratory system. Early stage of SARS-CoV-2 infection targets the nasal and bronchial epithelial cells and pneumocytes. Later, SARS-CoV-2 infects pulmonary capillary endothelial cells, triggering an inflammatory response. There may be activation of the coagulation cascade and disseminated intravascular coagulation	X-rays: ground glass opacities Blood gas: decrease pO2 [15]
Liver	Generally mild disease	Cause may be multifactorial: direct viral cytotoxicity, immune mediated, vascular changes due to coagulopathy, congestion following right sided heart failure, drug induced	Elevated bilirubin and liver enzymes [16]
Cardiac	Cardiomyopathy, heart failure Myocardial injury		ECG changes Elevated cardiac troponins Natriuretic peptides Elevated cardiac enzymes [17]
Kidney	Acute kidney injury Renal failure	Direct cytopathic effect Inflammatory mediated Complement activation Disseminated intravascular coagulation Rhabdomyolysis Organ cross talk e.g., hepatorenal syndrome Volume depletion	Elevated urea and creatinine Proteinuria, or albuminuria Abnormal blood electrolytes [18]
Vascular	Large vessels emboli Disseminated intravascular coagulation	Activation of renin angiotensin system cytokine storm	Elevated D dimers Low platelets Prolonged APTT and INR [19]
Neurological	Meningoencephalitis Seizures Cerebrovascular accidents Guillain-Barre syndrome Coma	Direct infection of neurons via ACE2 receptor Endothelial damage and hypercoagulation Immune mediated cytokine storm	CSF positive for SARS-CoV2 [20]

Table 7.2 Traditional diagnostic tests and their clinical utility

	RT-PCR	Antigen detection	Antibody detection
Specificity	Highly specific in *acute SARS-CoV-2	Specific RT-PCR is required in negative results	Variable - dependent on kit Indicates current or past infection
Technical requirements: Equipment Personnel Site	Requires expensive equipment and reagents		
	Highly skilled technicians	Minimal technical skills required	Minimal technical skills required
	Centralized laboratory testing	Can be done within hospital/ at point of care	Can be done within hospital/at point of care
Turnaround time	Extended	Short	Short
Advantages	Sensitive – Early diagnosis Specific	Scalable Can be automated Specific	Scalable Can be automated
Disadvantages	Non-automated Long Turnaround Times	Cross reactivity with related coronaviruses Poorer Sensitivity Variable performance depending on kit	Cross reactivity with related coronaviruses Variable performance depending on kit
Sample type	Naso/Oro pharyngeal Swab	Naso/Oro pharyngeal Swab Blood	Blood

[26]. This method is considered the gold standard. Its high sensitivity and specificity make it a good choice during the early phase of diagnosis when the viral load is low, with the diagnostic window preceding the onset of symptoms [25]. It does, however, require technical expertise and sophisticated equipment, requiring a laboratory environment to process the samples. The quality of the sample is imperative, and factors including sample type, collection, transportation, and storage can affect test performance [27]. These constraints result in a longer turnaround time which impacts service delivery and patient outcomes especially during periods of high demands like during an upward trend in infections, often termed a "wave."

There have been many advancements on the principles of RT-PCR which have significantly improved the utility of this test in diagnosis. These include techniques like the use of isothermal detection, next-generation sequencing (NGS), clustered regularly interspaced short palindromic repeats (CRISPR), and digital PCR. Isothermal amplification and detection techniques accumulate nucleic acids at a constant temperature, unlike traditional PCR which requires cyclic temperature changes. When combined with simpler readout methods and microfluidics, this has resulted in portable, accessible, and easy to use devices [26]. One such example is loop-mediated isothermal amplification (LAMP), which has been utilized widely in COVID-19 diagnostics. Advantages of this method include a greater yield than RT-PCR, it eliminates the need for sophisticated equipment, and it is cost-effective, easy to use, and accurate [2]. Studies evaluating its utility compared to the gold standard of RT-PCR have demonstrated excellent sensitivity of up to 97% [25].

NGS has been critical in the evolution of COVID-19 diagnostics. It allows for the description of the entire SARS-CoV-2 genome and therefore has been used to detect changes to the genome and identify emerging molecular variants. Its utility is confined to surveillance and epidemiology due the cost and technical requirements, but it has been key in managing the pandemic worldwide. Improvements in the NGS methods include amplicon-based metagenomics sequencing [26].

Clustered regularly interspaced palindromic repeats (CRISPR) technology is based on genome editing systems normally found in bacteria. It uses the collateral cleavage activity of endonucleases for viral nucleic acid detection [26]. Advantages of CRISPR over routine PCR-based methods include speed, sensitivity, specificity, and user-friendliness. The Sherlock CRISPR SARS-CoV-2 kit is the first CRISPR-based test to be used in patient testing that is US Food and Drug Administration (FDA)-approved [27].

Digital PCR is an improvement on routine PCR in which smaller volumes of sample are used. Droplet digital PCR uses the principles of micro-partitioning and ultra-dilutions. Each PCR reaction is conducted in multiple discrete replicate droplets and then detected by fluorescence [26].

Serological and immunology tests also have an important role in the COVID-19 healthcare response [28]. This can be by viral antigen detection or the patient's response to infection via antibody detection [29].

Tests that detect viral antigens can be utilized for diagnosis. Although these are less sensitive than the molecular testing, they have the potential to provide results quicker and cheaper and are, therefore, useful in settings where an urgent result is needed [30]. Antigen testing allows rapid identification of possible cases to help curb transmission. This includes fit for traveling and resumption of school or work, identifying patients who pose a risk of spreading infection, and in cases where laboratories are unable to keep up with the demand of molecular testing [25].

Antibody testing can be considered to provide indirect evidence of viral exposure at least within the past 1–2 weeks, and antibodies can persist up to 6 months [28, 30]. These rely on the detection of antibodies (IgG, IgA, IgM, and/or total antibodies), which may be specific for the receptor binding domain, nucleocapsid protein, spike protein, or both nucleocapsid and spike proteins of the SARS-CoV-2 virus. IgA levels increase early (within 1 week of symptoms) but usually decline rapidly within a few weeks. IgM levels also increase rapidly but decrease early on in the disease course. IgG levels can peak within 1–2 weeks but are valuable in that they can remain increased for up to 6 months [28]. Different assays detect any one or a combination of these antibodies, so it is vital to understand the characteristics of the test being used to aid interpretation of the findings.

While these antibody tests are inadequate for diagnosis, they can be useful in epidemiologic studies, surveillance, and vaccine development, as well as being useful for screening healthcare workers [27]. They allow for the evaluation of seroprevalence, which indicates if our control and containment measures have been effective [28]. The stability of human antibodies is thought to be superior to viral RNA, especially when considering pre-analytical issues like sample type, collection, transport, and storage [25]. This makes serological testing a good alternative in certain circumstances.

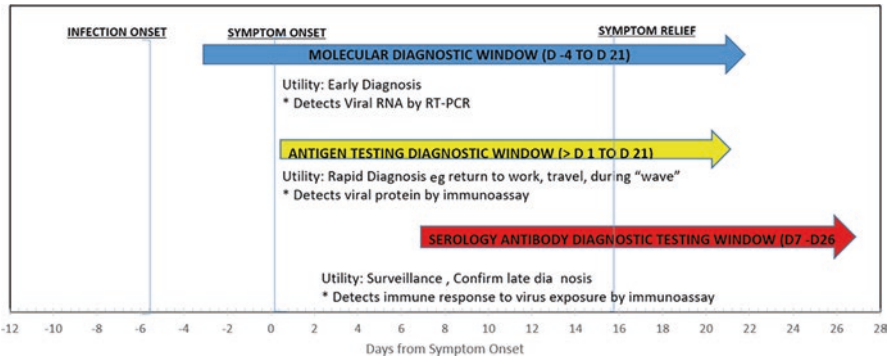

Fig. 7.1 Traditional tests: Diagnostic window and utility

Many methodologies have been approved by the WHO, ranging from manual assays to highly automated assays. These include enzyme-linked immunosorbent assays (ELISA), Western blot, immunofluorescence assays (IFAs), chemiluminescence assays (CLIAs), and protein microarrays [25, 26]. A benefit of antigen and antibody testing is the scalable nature of the testing which allows laboratories to meet the demands for testing during the different stages of a pandemic.

A limitation of the serological assays is related to the potential for cross-reactivity between antigens of SARS-CoV-2 and related coronaviruses like MERS-CoV [26]. The specificity has been reported to be from 96 to 100%. The lag between infection onset and the finding of a positive test limits its utility for early diagnostics. Studies to date have demonstrated that 5% of symptomatic and up to 40% of asymptomatic PCR-positive patients can remain seronegative [27]. It also remains to be determined what the effect of widespread vaccination drives will be on interpretation of the serology tests.

The clinical utility of these tests varies with time from infection, and this is summarized in Fig. 7.1.

4 COVID-19 Risk Biomarkers

The management of SARS-Cov-2-infected patients entails using biomarkers to aid in the diagnosis, prognostication, stratification, and therapeutic intervention, as well as monitoring and assessment of long-term COVID-19 sequelae. The mortality and severe morbidity associated with infection by this virus have been associated with many risk factors. A systematic review by Dessie et al. reported that chronic noncommunicable diseases, age, demographic variables, and lifestyle behavior were significant risk factors for severe disease and mortality [31]. Despite the earlier assertion that COVID-19 brings about a respiratory disease, the mortality has been linked to multiorgan dysfunction, which is secondary to viral infection and the immune response. Thus, early detection of organ dysfunction can help to mitigate

disease severity and aid in choices of therapeutic interventions for systemic SARS-CoV-2 disease.

The pathophysiology for the multiorgan failure with the SARS-Cov-2 virus is variable and is organ- and system-specific [32–35] (Table 7.3). Most organ failures are secondary to the overt immune response and direct infection of the cells by the virus. For instance, COVID-19-associated coagulopathy is associated with endothelial damage secondary to the inflammatory response. Some studies have indicated that the magnitude of the humoral response is proportional to disease severity.

The use of biomarker-based tests can aid in identifying organ involvement and can be used for risk stratification. The use of certain biomarkers has not been consistent across all studies. Hyperferritenima is marked by high levels of ferritin, a positive acute-phase protein associated with inflammatory disease, multiorgan dysfunction, and overt infections. Nonetheless, the evidence for its use in prognosticating patients has been inconsistent. Williams et al. reported that serum ferritin did not predict mortality in sepsis, although some studies looking at COVID-19 demonstrated that ferritin was a good prognostic marker [36, 37]. Most biomarkers follow different patterns depending on the phase/time since seroconversion. For instance, full blood count markers such as white cell, neutrophil, and platelet counts reach a nadir by day 8–9 of illness and subsequently improve. Therefore, these markers can be used in the first week of life to predict prognosis, and after 14 days, the increase in these markers can be used to assess recovery.

4.1 Risk Stratification and Prognostication of COVID-19 Patients

The limited hospital and critical care beds in resource-restricted African countries necessitated using prediction models to ensure timely intervention and deployment of true distributive justice. Disease stratification and prognostication are based on clinical presentation, medical history, bedside investigations (vitals and electrocardiography), radiological findings, and biochemical evidence of impending organ failure. Symptoms such as cyanosis, shortness of breath and altered mental status, and signs like SpO2 <94%, respiratory rate >30/min, systolic blood pressure <90 mm Hg, or other signs of shock or complications are associated with high risk. High-risk and severe disease patients require urgent hospitalization, and critical care is needed in extreme cases. Many institutions, including the WHO, have provided algorithms to ensure quick and efficient patient triaging during a crisis. A scoring system is mandatory to assist resource allocation in a resource-limited setting.

In South Africa, the sequential organ failure assessment (SOFA) score was adopted in many high care and intensive care units. This scoring system determines the level of organ dysfunction and mortality risk in ICU patients. The score was first reported by Vincent et al. and has 0 to 4 points assigned to each of 6 organ systems based on several analytes and the Glasgow coma scale [38]. Thus, the SOFA score

Table 7.3 Pathophysiology of multiorgan failure associated with the SARS-Cov-2 virus

Organ/system	Pathogenesis	Biomarkers
Respiratory	Bronchopneumonia and acute respiratory distress syndrome are common pulmonary presentations. The ventilation and perfusion abnormalities are due to the following Direct viral infection of the bronchial epithelial cells and the alveolar type I and type II pneumocytes Inflammatory response Activation of coagulation and formation of microthrombi Vascular permeability due to lack of ACE 2 receptors and inflammatory response Atelectasis, Pulmonary oedema and fibrosis	Blood gas: pO_2, pCO_2, bicarbonate Neuron specific enolase Lactate dehydrogenase Metabolomics markers: peroxisome proliferator-activated receptors PPAR, D-arginine, D-ornithine, TRP, alpha-linoleic [32]
Hematological	COVID-associated lymphocytopenia is secondary to the direct infection of cells by the virus via the ACE receptors, resulting in cell death. The cytokine storm is also linked to cell apoptosis. Cytokine storm-induced atrophy of lymphoid organs and reduced lymphocyte proliferation due to lactic acidosis	High Neutrophil: lymphocyte ratio Peak platelet/lymphocyte ratio Thrombocytopenia Lymphopenia Neutrophilia [33]
Coagulation	The patients are prone to venous thromboembolic events (VTE) and disseminated intravascular coagulopathy (DIC). Endothelial dysfunction is secondary to the virus binding to the ACE 2 receptor and the release of inflammatory mediators, which result in increased blood viscosity.	Marked prolongation of PT and aPTT elevated d-dimer Elevated fibrinogen [34]
Inflammation	The virus triggers host and innate immunity responses upon entry into the host cells. Neutrophils are recruited, and these release cytokines. The cytokine response leads to a wide spectrum of systems dysfunctions	Elevated CRP Elevated IL-6 Neutrophilia Elevated ESR (erythrocyte sedimentation rate) Elevated serum ferritin Elevated PCT Omics: microRNA
Cardiac	Cardiac dysfunction is attributed to the direct viral invasion of cardiomyocytes, secondary to VTE, and the immune-mediated response. The cardiac complication noted are namely myocarditis, cardiac failure, cardiomyopathy, acute myocardial infarction, arrhythmias and cardiac arrest	Elevation in cardiac Troponin I and T Elevation on NT ProBNP /or BNP [35]
Musculoskeletal	Direct viral infection of cells and immune response affect the myocytes. Severe myositis	Creatine-kinase (CK) Myoglobin

(continued)

Table 7.3 (continued)

Organ/system	Pathogenesis	Biomarkers
Hepatic	Direct virus infection of hepatocytes, endothelial damage secondary to cytokine storm, tissue hypoxia, and VTE result in hepatobiliary dysfunction. The decreased synthetic function increased capillary permeability and increased turnover of albumin, resulting in hypoalbuminemia,	Elevated transaminases: AST and ALT Hypoalbumin Elevated lactate dehydrogenase Elevated bilirubin
Renal	Kidney damage is mainly an acute kidney injury that may lead to chronic nephropathy. The pathophysiology of kidney dysfunction in SARS-CoV-2 is due to direct nephron infections, endothelial vasculitis, VTE and hypoxia of the kidney cells	Creatinine Neutrophil gelatinase-associated lipocalin (NGAL), Cystatin C, Kidney injury molecule-1 (KIM-1), Urine protein creatinine clearance
Electrolytes	The reduction in aldosterone activity is secondary to drugs such as chloroquine. Hypokalemia is secondary to GIT losses, increased angiotensin II and kidney disease. Hyponatremia is due to SIADH secondary to cytokines noted in pneumonia and ARDS Hypocalcaemia is attributed to lower intracellular calcium, as two Ca^{2+} ions bind to the SARS-COV-2 fusion peptide	Hyponatremia/hypernatremia Hypokalemia Hypocalcaemia

ranges from 0 to 24 points, and higher scores indicate worse organ function. However, this scoring system was deemed ineffective during the early phase of pandemic, as COVID-19 was hypothesized to be a single-organ dysfunction disease.

In developing countries, the need to decide the level of care is critical due to the limited availability of critical care units. The scoring tools used to evaluate the patients and decide the patient therapeutic plans require a number of biochemical and hematological analytes. These can be measured on point of care devises thus allowing for rapid triage of patients.

4.2 Cytokine Testing

The SARS-CoV-2 infection triggers both innate and adaptive immune responses, with a characteristically excessive pro-inflammatory response of the innate immune system [39]. Adding on to this, the dysregulated host response of the adaptive immune system can lead to tissue damage. As a result, a massive amount of cytokines and chemokines are released, mainly interleukins 2 and 6 (IL-2 and IL-6) as well as tumor necrosis factor (TNF-α). This cytokine storm is a hallmark of severe SARS-CoV-2 infection, and the cytokines released can cause endothelial damage, hypercoagulability, alveolar damage, and multiorgan failure [40].

Table 7.4 Clinical utility of IL-6

Clinical utility	IL-6 levels
Assessment of severity	Increased
Response to therapy	Decreases
Predicting outcome	Variable
Multisystem inflammatory syndrome in children	Increased
Cytokine storm	Increased

IL-6 is a circulating multifunctional 26 kDa protein consisting of 26 amino acids. It has a pro-inflammatory function and may be acutely elevated in COVID-19 patients. IL-6 stimulates production of acute phase proteins, acts as a maturing agent for B lymphocytes, stimulates the synthesis of immunoglobulins, induces proliferation of T cells, and activates natural killer cells. In COVID-19, IL-6 levels follow a temporal course with a peak between 7–14 days post-infection [41]. The levels of IL-6 with other cytokines may remain elevated for 4 weeks post-infection in severe cases [42]. IL-6 levels can be used for prognostication, with higher IL-6 baseline results correlating with severe, bilateral interstitial involvement, in keeping with other acute inflammatory markers [43]. IL-6 may also be useful in monitoring therapeutic response [44] (Table 7.4).

Another cytokine of clinical importance in COVID-19 is TNF-α. Active TNF-α is a pro-inflammatory homotrimer of 17 kDa polypeptides with a total molecular weight of 52 kDa. It is produced by activated macrophages, monocytes, T lymphocytes, and natural killer cells. TNF-α mediates and regulates development of the immune system, proliferation, cell survival signaling, and metabolic processes, as well as apoptosis [45]. Elevated serum TNF-α was found in patients with severe COVID-19 and in those admitted to the ICU and with poor clinical outcomes [46]. Together with measurements of IL-6, TNF-α was shown to be predictive of COVID-19 disease severity and mortality. The role of TNF-α in disease pathogenesis has also highlighted a potential role for anti-TNF-α therapeutics. This therapy aims to reverse TNF-induced immunopathology to improve the prognosis of COVID-19 patients. Therefore, measurements of TNF-α have a potential role in monitoring disease severity and prognosticating in COVID-19 patients [47]. TNF-α can be measured using flow cytometry, ELISA, and chemiluminescence as well as by microfluidic methods.

IL-10 is produced by regulatory T cells and T helper 1 cells for immunoregulation and as part the inflammatory response. IL-10 may be pro-inflammatory and immune-activating in COVID-19 pathogenesis. Studies have shown that patients with elevated IL-6 also have higher circulating levels of IL-10 and TNF-α. This relationship was observed in COVID-19 patients with severe disease and positively correlated with mortality. Therefore, IL-10 has been identified as a disease severity and mortality biomarker in COVID 19. Similar to TNF-α, IL-10 is a potential target for therapeutic intervention to reduce mortality in SARS-CoV-2 infections [47].

The use of point of care testing (POCT) detection of cytokines has become imperative in the context of COVID-19 as it offers rapid assessment of disease severity. The POCT cytokine measurement allows for early diagnosis and monitoring of the cytokine storm in particular. Cytokines in the context of COVID-19 have been measured mainly in serum or plasma in clinical practice [48]. However, they can also be measured in matrices such as whole blood, interstitial fluid, and cerebrospinal fluid. Methodologies that are in use for cytokine detection currently are immunoassays, including electrochemiluminescent multiplex immunoassays and enzyme-linked immunosorbent assays (ELISA). Immunoassays are often automated in a central laboratory and thus not suitable for near patient testing. Also, the interpretation of results is challenging due to differences in method standardization and potential errors due to the presence of cytokine binding proteins and variable cytokine forms [49].

Commonly available POCT designs have been used in cytokine measurements. These include colorimetric lateral flow assays, fluorescence lateral flow assays, electrochemical impedance spectrometry spectroscopy, and field effect transistors [49]. Improvements on POCT devices have been made possible by the use of biosensors for the detection of cytokines. These use biochemical reactions and bioelectronic technologies for quick and reliable detection of pathogens [26]. Biosensors also allow for improved analytical sensitivity, analysis time, and smaller sample volume and offer multiplex detection [49].

5 The Use of Multiomics in Understanding SARS-CoV-2 Infection

Multiomic technologies have been used to describe the viral genotype and the pathogenesis of SARS-CoV-2. For example, NGS allowed the original identification of SARS-CoV-2 as well as the origins of the virus [50, 51]. It was then possible to develop RT-PCR tests for diagnostic use. This was subsequently followed by parallel detection of SARS-CoV-2 and other viruses that cause respiratory tract infections [52, 53].

The areas of research included in the omics field include proteomics, transcriptomics, genomics, metabolomics, lipidomics, and epigenomics, which allow parallel and comprehensive analyses of proteins, RNA, genes, metabolites, lipids, and methylated DNA or modified histone proteins in chromosomes, respectively.

Genomics has enabled understanding and diagnosis of SARS-CoV-2 from first identification to current identification of mutant strains. The African continent was not left out in these developments, as there is now genomic-based surveillance for COVID-19 informing diagnostic tests and vaccines. This surveillance was based on genome sequencing of the SARS-CoV-2 virus and has enabled Kenya and South Africa to delineate imported cases involving community transmission. These findings were also crucial to direct public health policies and containment responses to the COVID-19 pandemic in the early stages [54].

Table 7.5 Postulated transcriptome-based immune profiling implications

Method	Findings	Profiling implication
Viral transcriptome analysis	41 sites of RNA 5 – methyl cytosine modification	Instability of viral RNA's and immune escape [55]
Single cell transcriptomes	Upregulated Squamous epithelial cells ANXA1, S100A8 and S100A9 with upregulated Neutrophil and Macrophages FPR1 and TLR4	Clarify immune characteristics and mechanisms resulting in the cytokine storm [56]
Sequencing non coding RNA and mRNA	miR-146a-5p; miR-21-5p; miR-142-3p; miR-15b-5p were related to the severity of COVID 19	Heterogeneity of COVID 19 and classifying COVID 19 severity [57]
Plasma multiomics	Dysfunctional $S100^{high}$ $HLA\text{-}DR^{low}$ monocyte subpopulation is related to COVID 19 severity	Differentiation between levels of severity in COVID 19 [58]

Transcriptomics has been enabled by the progression in sequencing technology. The genomic transcriptome analysis of the SARS-CoV-2 has elucidated gene expression information of the virus and an understanding interaction of the virus with the host. Importantly, it has allowed for immune profiling as illustrated in Table 7.5 [55–58] and for understanding the pathogenesis as illustrated in Table 7.6 [59–62].

Metabolomics which studies small molecules with a relative molecular weight of less than 1000 Da has also been applied in the study of COVID-19. Through quantitative analysis of metabolites, their mechanistic relationship with physiological and pathological changes has been explored. Techniques used include ultraperformance liquid chromatography/tandem mass spectrometry and multiomic approaches, such as combined metabolomic and lipidomic profiling. One study found that a plasma lipid monosialodihexosyl ganglioside (GM3) was inversely associated with CD4+ T cell count in COVID-19 patients [63]. The study suggested that GM3-rich exosomes may be involved in the pathogenesis of COVID-19 by affecting microenvironmental homoeostasis. This study also identified an association between GM3-enriched exosomes and COVID-19 severity. Such findings can inform development of diagnostic assays to detect small changes in GM3 with the potential value for diagnosing and classifying COVID-19 patients. In the future, it is anticipated that omics platforms will inform practice through diagnostics, prognostication, surveillance, and clinical decision making, which are all relevant to improving COVID-19 disease outcomes.

6 Conclusions and Future Perspectives

The complexity and heterogeneity of COVID-19 infection is challenging for diagnostic sciences. However, there has been rapid progress from identification of the virus and diagnosis based on RT-PCR, through point of care tests and management

Table 7.6 Pathogenic mechanisms generated by multiomic studies

Method	Findings	Profiling implication	Reference
Multiorgan proteomic profile; Autopsies analysis of 5336 protein molecules	Upregulated cathepsin L1 in the lungs, dysregulation of factors related to hypoxia, angiogenesis, coagulation and fibrosis in multiple organs	Differentially expressed proteins may be candidate biomarkers for diagnosis and prognosis of severe COVID -19 cases	[59]
Model based on machine learning: Prioritization of optimal biomarker Combinations for COVID-19 (POC-19)	1. Four protein biomarkers were identified as classifiers include orosomucoid-1/alpha-1-acid glycoprotein-1 (ORM1/AGP1), Alpha-1-acid glycoprotein 2 (ORM2), fetuin-B (FETUB), and cholesteryl ester transfer protein (CETP) as classifies and 2. Outcome markers identified were zinc-a2-glycoprotein 1 (AZGP1), ORM2, and complement factor I (CFI) alone or in combination, 3. Markers predicting recovery include combination of serine proteinase inhibitor A3/a1- antichymotrypsin (SERPINA3/ACT), lymphocyte cytosolic protein 1/L-plastin (LCP1/LPL), and peptidase inhibitor 16 (PI16)	COVID 19 patient classification, disease progression prediction and prediction of recovery. Investigation in a large cohort is required	[60]
Time resolved proteomics using Flow chromatography and mass spectrometry, SWATH-MS quantitative and deep-neural network methods	Dynamic changes in markers reflecting progression of disease: immuno-inflammatory mediators CD44 and B2 M, complement cascade components CFD and CFHRs, coagulation components HRG and PLG, apolipoprotein APOA2, APOC3 and angiotensin (AGT), as well as the organ dysfunction indicators NT-proBNP and troponin	Prediction model of disease progression, and oxygen therapy intervention, Identify early infected individuals and direct risk stratification	[61]
Ultra-high throughput proteomic assay using short-gradient highflow liquid chromatography (LC)	27 proteins identified that are closely associated with IL-6-mediated proinflammatory signaling	Valuable biomarkers of disease severity	[62]

of risk factors for severe disease to the use of multiomics. The application of many of these tests in resource poor countries remains suboptimal. Current point of care COVID-19 tests may not perform well early in the course of infection. Improved and affordable diagnostics are needed in resource constrained countries. Some tests such as IL-6 are used to predict disease severity and response to treatment and are

currently not widely available in Africa. The dream would be to have a widely available and easily accessible point of care for multi-array diagnosis followed by tests for risk stratification. The principles established from the COVID-19 pandemic should guide the future of pandemic diagnostics.

References

1. GenBank: MN908947.3. Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome. https://www.ncbi.nlm.nih.gov/nuccore/MN908947
2. World Health Organization (2020) Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases; Interim guidance. https://www.who.int/publications/i/item/10665-331501
3. Zhu N, Zhang D, Wang W, et al (2020) A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med 382(8):727–733
4. Li W, Moore MJ, Vasilieva N, et al (2003) Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 426(6965):450–454
5. Walls AC, Park YJ, Tortorici MA, et al (2020) Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell 181(2):281–292.e286
6. Lan J, Ge J, Yu J, et al (2020) Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 581(7807):215–220.
7. Lu J, Gu J, Li K, et al (2020) COVID-19 Outbreak Associated with Air Conditioning in Restaurant, Guangzhou, China, 2020. Emerg Infect Dis 26(7):1628–1631
8. World Health Organization (2020) Modes of Transmission of Virus Causing COVID-19: Implications for IPC Precaution Recommendations. https://www.who.int/news-room/commentaries/detail/modes-of-transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations
9. Arons MM, Hatfield KM, Reddy SC, et al (2020) Presymptomatic SARS-CoV-2 Infections and Transmission in a Skilled Nursing Facility. N Engl J Med 382(22):2081–2090
10. He X, Lau EHY, Wu P, et al (2020) Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med 26(5):672–675
11. Ganyani T, Kremer C, Chen D, et al (2020) Estimating the generation interval for coronavirus disease (COVID-19) based on symptom onset data, March 2020. Euro Surveill 25(17):2000257. https://doi.org/10.2807/1560-7917.ES.2020.25.17.2000257
12. Fenizia C, Biasin M, Cetin I, et al (2020) Analysis of SARS-CoV-2 vertical transmission during pregnancy. Nat Commun 11(1):5128. https://doi.org/10.1038/s41467-020-18933-4
13. Centers for Disease Control and Prevention (2020) COVID-19: Symptoms. https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html
14. Chen N, Zhou M, Dong X, et al (2020) Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 395(10223):507–513
15. Synowiec A, Szczepański A, Barreto-Duran E, et al (2021) Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): a Systemic Infection. Clin Microbiol Rev 34(2):e00133–20. https://doi.org/10.1128/CMR.00133-20
16. Nardo AD, Schneeweiss-Gleixner M, Bakail M, et al (2021) Pathophysiological mechanisms of liver injury in COVID-19. Liver Int 41(1):20–32
17. Tajbakhsh A, Gheibi Hayat SM, Taghizadeh H, et al (2021) COVID-19 and cardiac injury: clinical manifestations, biomarkers, mechanisms, diagnosis, treatment, and follow up. Expert Rev Anti Infect Ther 19(3):345–357

18. Ahmadian E, Hosseiniyan Khatibi SM, Razi Soofiyani S, et al (2021) Covid-19 and kidney injury: Pathophysiology and molecular mechanisms. Rev Med Virol 31(3):e2176. https://doi.org/10.1002/rmv.2176
19. Lazzaroni MG, Piantoni S, Masneri S, et al (2021) Coagulation dysfunction in COVID-19: The interplay between inflammation, viral infection and the coagulation system. Blood Rev 46:100745. https://doi.org/10.1016/j.blre.2020.100745
20. Aghagoli G, Gallo Marin B, Katchur NJ, et al (2021) Neurological Involvement in COVID-19 and Potential Mechanisms: A Review. Neurocrit Care 34(3):1062–1071
21. World Health Organization (2022) Coronavirus Dashboard. https://covid19.who.int/
22. Musa HH, Musa TH, Musa IH, et al (2021) Addressing Africa's pandemic puzzle: Perspectives on COVID-19 transmission and mortality in sub-Saharan Africa. Int J Infect Dis 102:483–488
23. Western Cape Department of Health in collaboration with the National Institute for Communicable Diseases, South Africa (2021) Risk Factors for Coronavirus Disease 2019 (COVID-19) Death in a Population Cohort Study from the Western Cape Province, South Africa. Clin Infect Dis 73(7):e2005-e2015
24. Jassat W, Cohen C, Tempia S, et al (2021) Risk factors for COVID-19-related in-hospital mortality in a high HIV and tuberculosis prevalence setting in South Africa: a cohort study. Lancet HIV 8(9):e554-e567
25. Younes N, Al-Sadeq DW, Al-Jighefee H, et al (2020) Challenges in Laboratory Diagnosis of the Novel Coronavirus SARS-CoV-2. Viruses 2(6):582. https://doi.org/10.3390/v12060582
26. Sharma A, Balda S, Apreja M, et al (2021) COVID-19 Diagnosis: Current and Future Techniques. Int J Biol Macromol 193 (Pt B):1835–1844
27. Wang YC, Lee YT, Yang T, et al (2020) Current diagnostic tools for coronaviruses-From laboratory diagnosis to POC diagnosis for COVID-19. Bioeng Transl Med 5(3):e10177. https://doi.org/10.1002/btm2.10177
28. Ong DSY, Fragkou PC, Schweitzer VA, et al (2021) How to interpret and use COVID-19 serology and immunology tests. Clin Microbiol Infect 27(7):981–986.
29. Wong RSY (2021) COVID-19 testing and diagnosis: A comparison of current approaches. Malays J Pathol 43(1):3–8
30. Peeling RW, Heymann DL, Teo YY, Garcia PJ (2022) Diagnostics for COVID-19: moving from pandemic response to control. Lancet 399(10326):757–768
31. Dessie ZG, Zewotir T (2021) Mortality-related risk factors of COVID-19: a systematic review and meta-analysis of 42 studies and 423,117 patients. BMC Infect Dis 21(1):855. https://doi.org/10.1186/s12879-021-06536-3
32. Saccon E, Bandera A, Sciume M, et al (2021) Distinct Metabolic Profile Associated with a Fatal Outcome in COVID-19 Patients during the Early Epidemic in Italy. Microbiol Spectr 9(2):e0054921. https://doi.org/10.1128/Spectrum.00549-21
33. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, et al (2020) Hematological findings and complications of COVID-19. Am J Hematol 95(7):834–847
34. Rahi MS, Jindal V, Reyes SP, et al (2021) Hematologic disorders associated with COVID-19: a review. Ann Hematol 100(2):309–320
35. Long B, Brady WJ, Koyfman A, Gottlieb M (2020) Cardiovascular complications in COVID-19. Am J Emerg Med 38(7):1504–1507
36. Williams V, Menon N, Bhatia P, et al (2020) Serum Ferritin Predicts Neither Organ Dysfunction Nor Mortality in Pediatric Sepsis Due to Tropical Infections. Front Pediatr 8:607673. https://doi.org/10.3389/fped.2020.607673
37. Sang L, Teng W, Zhao M, et al (2022) Association between serum ferritin and outcomes in critically ill patients: a retrospective analysis of a large intensive care unit database. Chinese Medical Journal. https://doi.org/10.1097/CM9.0000000000002019
38. Vincent JL, Moreno R, Takala J, et al (1996) The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med 22(7):707–710

39. Catanzaro M, Fagiani F, Racchi M, et al (2020) Immune response in COVID-19: addressing a pharmacological challenge by targeting pathways triggered by SARS-CoV-2. Signal Transduct Target Ther 5(1):84. https://doi.org/10.1038/s41392-020-0191-1
40. Ragab D, Salah Eldin H, Taeimah M, et al (2020) The COVID-19 Cytokine Storm; What We Know So Far. Front Immunol 11:1446. https://doi.org/10.3389/fimmu.2020.01446
41. Chen R, Sang L, Jiang M, et al (2020) Longitudinal hematologic and immunologic variations associated with the progression of COVID-19 patients in China. J Allergy Clin Immunol 146(1):89–100
42. Zhao Y, Qin L, Zhang P, et al (2020) Longitudinal COVID-19 profiling associates IL-1RA and IL-10 with disease severity and RANTES with mild disease. JCI Insight 5(13):e139834. https://doi.org/10.1172/jci.insight.139834
43. Liu T, Zhang J, Yang Y, et al (2020) The role of interleukin-6 in monitoring severe case of coronavirus disease 2019. EMBO Mol Med 12(7):e12421. https://doi.org/10.15252/emmm.202012421
44. Zeng F, Huang Y, Guo Y, et al (2020) Association of inflammatory markers with the severity of COVID-19: a meta-analysis. Int J Infect Dis 96:467–474
45. Varfolomeev E, Vucic D (2018) Intracellular regulation of TNF activity in health and disease. Cytokine 101:26–32
46. Sen A, Nigam A, Vachher M (2022) Role of polypeptide inflammatory biomarkers in the diagnosis and monitoring of COVID-19. Int J Pept Res Ther 28(2):59. https://doi.org/10.1007/s10989-022-10366-5
47. Udomsinprasert W, Jittikoon J, Sangroongruangsri S, Chaikledkaew U (2021) Circulating Levels of Interleukin-6 and Interleukin-10, But Not Tumor Necrosis Factor-Alpha, as Potential Biomarkers of Severity and Mortality for COVID-19: Systematic Review with Meta-analysis. J Clin Immunol 41(1):11–22
48. Atallah J, Archambault D, Randall JD, et al (2022) Rapid Quantum Magnetic IL-6 Point-of-Care Assay in Patients Hospitalized with COVID-19. Diagnostics (Basel) 12(5):1164. https://doi.org/10.3390/diagnostics12051164.2022;12:1164
49. Liu G, Jiang C, Lin X, Yang Y (2021) Point-of-care detection of cytokines in cytokine storm management and beyond: Significance and challenges. View (Beijing) 2(4):20210003. https://doi.org/10.1002/VIW.20210003
50. Zhou P, Yang XL, Wang XG, et al (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579(7798):270–273
51. Wu F, Zhao S, Yu B, et al (2020) A new coronavirus associated with human respiratory disease in China. Nature 579(7798):265–269
52. Corman VM, Landt O, Kaiser M, et al (2020) Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 25(3):2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045
53. Yelagandula R, Bykov A, Vogt A, et al (2020) SARSeq, a robust and highly multiplexed NGS assay for parallel detection of SARS-CoV2 and other respiratory infections. medRxiv. https://doi.org/10.1101/2020.10.28.20217778
54. Tessema SK, Inzaule SC, Christoffels A, et al (2020) Accelerating genomics-based surveillance for COVID-19 response in Africa. Lancet Microbe 1(6):e227-e228
55. Kim D, Lee JY, Yang JS, et al (2020) The Architecture of SARS-CoV-2 Transcriptome. Cell 181(4):914–921.e10
56. Ren X, Wen W, Fan X, et al (2021) COVID-19 immune features revealed by a large-scale single-cell transcriptome atlas. Cell 184(23):5838. https://doi.org/10.1016/j.cell.2021.10.023
57. Tang H, Gao Y, Li Z, et al (2020) The noncoding and coding transcriptional landscape of the peripheral immune response in patients with COVID-19. Clin Transl Med 10(6):e200. https://doi.org/10.1002/ctm2.200
58. Su Y, Chen D, Yuan D, et al (2020) Multi-Omics Resolves a Sharp Disease-State Shift between Mild and Moderate COVID-19. Cell 183(6):1479–1495.e20

59. Nie X, Qian L, Sun R, et al (2021) Multi-organ proteomic landscape of COVID-19 autopsies. Cell 184(3):775–791 e714
60. Shu T, Ning W, Wu D, et al (2020) Plasma Proteomics Identify Biomarkers and Pathogenesis of COVID-19. Immunity 53(5):1108–1122.e5
61. Demichev V, Tober-Lau P, Lemke O, et al (2021) A time-resolved proteomic and prognostic map of COVID-19. Cell Syst 2(8):780–794.e7
62. Messner CB, Demichev V, Wendisch D, et al (2020) Ultra-High-Throughput Clinical Proteomics Reveals Classifiers of COVID-19 Infection. Cell Syst 11(1):11–24.e4
63. Stencel-Baerenwald JE, Reiss K, Reiter DM, et al (2014) The sweet spot: defining virus–sialic acid interactions. Nat Rev Microbiol 12(11):739–49