

Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia  
 Open Access Macedonian Journal of Medical Sciences. 2020 Oct 26; 8(T1):371-381.  
<https://doi.org/10.3889/oamjms.2020.5296>  
 eISSN: 1857-9655  
**Category:** T1 - Thematic Issue "Coronavirus Disease (COVID-19)"  
**Section:** Narrative Review Article



# Overview of Pathology and Laboratory Features of COVID-19

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## Abstract

**BACKGROUND:** The third identified severe respiratory disease in the past two decades and the first to result in a pandemic is the coronavirus disease 2019 (COVID-19) specifically caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).

**AIM:** The aim of this study was to provide an overview of the basic pathology and diagnostic laboratory features of COVID-19.

**METHODS:** This review involved search of literatures on PubMed, Science alert, Medline, and Advanced Google search using the keywords "SARS-CoV2," "Coronavirus" along with "pathology of COVID-19" and "diagnosis of COVID-19" with related articles pooled, relevant information extracted, and properly referenced.

**RESULTS:** The recommended method of diagnosis is by nucleic acid testing of the viral ribonucleic acid in which real-time reverse-transcription polymerase chain reaction followed by nucleic acid sequencing when required is performed while some serologic techniques developed include enzyme-linked immunosorbent assay, immunochromatographic lateral flow assay, neutralization bioassay, and specific chemosensors to detect the immunoglobulin M and immunoglobulin G antibodies produced, although sole use of serologic tests is highly discouraged by monitoring agencies. The hematological features display leukocytosis with lymphocytopenia, eosinopenia, reduced procalcitonin along with increased D-dimer, C-reactive protein, serum amyloid A, fibrin, fibrin degradation products, and some inflammatory markers such as interleukin (IL)-2, IL-6, IL-7, IL-10, granulocyte colony-stimulating factor, MCP10, interferon gamma-induced protein 10, and tumor necrosis factor- $\alpha$ . The pathologic presentations include pleurisy, lung consolidation, pulmonary edema and pericarditis along with other features of acute respiratory syndrome, myocardial injury, and acute kidney injury.

**CONCLUSION:** There are highly efficacious and reliable methods of diagnosis of COVID-19 which also determine the stage and severity of the condition, and these methods are performed in line with specific clinical presentations. However, more studies are required to identify the capabilities, characteristics, and tendencies of this novel virus.

## Introduction

Coronavirus disease 2019 (COVID-19), a latest outbreak proclaimed as a pandemic by the World Health Organization (WHO), is a diseased condition resulting from infection of the severe acute respiratory syndrome corona virus-2 (SARS-CoV-2) [1]. The disease was named on February 11, 2020, by the WHO and subsequently declared a pandemic, on March 11, 2020 [2], [3], when it was recorded that there were more than 118,000 cases in 114 countries and 4291 deaths as a result of its rapid spread through human to human transmission by aerosol droplets to all continents of human existence with 13-fold increase [4]. COVID-19 is the third identified severe respiratory illness caused by the coronavirus in the past two decades and the first to cause a pandemic [4]. Others such as SARS and Middle East respiratory syndrome (MERS) have been earlier identified possessing similar susceptibility [5].

SARS-CoV-2 was classified by the International Committee on Taxonomy of Viruses (ICTV) as a newly identified strain of ribonucleic acid (RNA) viruses

which had not been previously isolated in humans until recently when it was discovered to be transmitted from human to human resulting in series of respiratory tract disorders, ranging from various levels of severity which commenced in Wuhan, China [6], [7], [8], [9]. The complete genome sequencing information from two assemblage revealed that the SARS-CoV-2 comprises six major open reading frames and shares approximately 80% of similarity with SARS-CoV, but 98.65% nucleotide identity to partial RNA-dependent RNA polymerase (RdRp) gene and 96.2% identity to RaTG13 of SARS-related CoronaVirus family found in bats, respectively [6], [10], [11], [12].

Globally, as at July 17, 2020, there have been 13,616,593 confirmed cases of COVID-19, including 585,727 deaths with 7,154,840; 3,008,972; 1,346,792; 1, 308,441; 543,122; and 253,495 confirmed cases in America, Europe, Eastern Mediterranean, South-East Asia, Africa, and Western Pacific continents, respectively [13], [14]. Despite the documented comparatively low mortality, the transmissibility of COVID-19 is observably elevated [15], [16]. This transmissibility potential is the  $R_0$  value which

is a pointer of the viral ability to transmit, thereby representing the average figure of new infections produced by an infectious person in a uninfected populace. When  $R_0 > 1$ , this indicates that there is the possibility of increased infection while  $R_0 < 1$  indicates the fading off of transmission. An essential concept of infectious disease epidemiology is the reproduction number which indicates the menace associated with the endemic spread of an infectious agent [17]. The basic reproduction number ( $R_0$ ) of COVID-19 has been estimated to range from 1.4 to 6.47, while some studies have shown the  $R_0$  value to be  $>3$  [16], [17], [18].

The COVID-19 is characterized by various degenerative symptoms such as pyrexia, dry or hoarse cough, sputum, gasping or cessation of breath, loss of taste sensation and/or smell, exhaustion, and lymphopenia in infected persons with the infections causing viral pneumonia which may lead to SARS and mortality in more severe cases or persons with underlining conditions such as diabetes and hypertension [6], [7], [8], [9], [19].

With the current trend in the management, epidemiology and occurrences in this pandemic, this review attempts to appraise the pathology, diagnosis, and laboratory features of COVID-19.

## Methodology

This review involved search of the literatures on PubMed, Medline, Science alert, and Advanced Google search using the keywords "SARS-CoV2," "Coronavirus" along with "pathology of COVID-19" and "diagnosis of COVID-19" in this text with related articles pooled and relevant information extracted, and properly referenced. From the search, we obtained 205 (220) articles out of which relevant information for this study were specifically extracted from 96 of the publications for this article.

### **Pathogenesis of COVID-19 infection**

Coronaviruses are a family of enveloped single-stranded RNA viruses with zoonotic features, and clinical signs analogous to the regular flu, neurological, severe respiratory, hepatic, and gastrointestinal symptoms [4], [20]. The six other identified coronaviruses which can infect humans aside the SARS-CoV-2 [21], are HCoV-229E, HCoV-OC43, SARS-CoV, HCoV-NL63, HCoV-HKU1, and MERS-CoV [6], [21], [22], [23]. They have created two major pandemics in the past 20 years which are the SARS [24] and MERS [11], [21], [25]. In detection of the major source of COVID-19, China Centre for Disease Control (CDC) analysts pointed that it originated from wild animals that are sold in the Wuhan local market (Chinese CDC, 2020). The

researchers obtained and analyzed throat swab, lung fluid, and blood samples of 15 patients infected with the virus. The analyses discovered that the virus-specific nucleic acid sequences in the sample differ from those of previously known human coronavirus (hCoV) species displaying that SARS-CoV-2 has similar properties to some of the beta ( $\beta$ ) coronaviruses genera identified in bats [8], [11], [26], [27], among the SARS/SARS-like CoV group [11], [21].

The outcome of the next-generation sequencing performed by these researchers indicated that the features of SARS-CoV-2 differ from SARS-CoV (with about 79% sequence identity) and MERS-CoV (with about 50% sequence identity) than from the group of two bat-derived SARS-such as coronaviruses – bat-SL-CoVZC45 (with 87.9% sequence identity) and bat-SL-CoVZXC21 (with 87.2% sequence identity) [28]. Studies also reported that COVID-19 S-protein supported strong relationship with human angiotensin converting enzyme 2 (ACE2) molecules in spite of the variation in its sequence with that of SARS-CoV [11], [21], [29]. Although detailed pathogenesis of this novel infection is emerging day by day, the mechanism of infection has been described to entail the adhesion of the viral spike protein to the ACE2 molecule of the type II pneumocyte receptor (a precursor of type I pneumocyte) and the virion is endocytosed by the help of a serine protease TMPRSS2. On entering the cell, the virion RNA is released and translated by the cells machinery into non-structural polyproteins that are cleaved into proteases, which are RNA-dependent, RNA polymerases, and structural proteins. Then replication complex forms to produce more RNA with viral proteins and RNA assembling into a newly manufactured virion in the Golgi consequently releasing the virion to infect new Type II pneumocytes [30]. This, in turn, result in aggressive inflammatory response leading to vascular endothelial dysfunction and subsequently endotheliopathy, with sepsis-induced coagulopathy (SIC) as described by the international society for thrombosis (ISTH) [31], [32]. The SIC in COVID-19 could occur by activation of coagulation through several procoagulant pathways such as viral adhesion on an ACE2 receptor present on endothelial cells, inflammatory cell infiltration coupled with apoptosis of endothelial cells, and microvascular prothrombotic effects [33] which explains the myocardial ischemia and increasing reports of macro- and micro-thromboembolic complication in some patients [33], [34], [35].

Furthermore, the pathobiology of this process has been further classified into three stages [36] which are: The asymptomatic state occurring within 1–2 days of infection whereby the inhaled virus will bind the epithelial cells of the nasal cavity through the assistance of the ACE2 receptors with the consequent initiation of a limited innate immune response [37], [38]; the next stage is symptomatic phase characterized by the upper and conducting airway response occurring within few days of infection triggering a more robust innate immune response evidenced with the presence

of beta and lambda interferons, especially C-X-C motif chemokine ligand 10, an excellent interferon responsive gene to the alveolar type 2 cell in SARS and influenza infection. Finally, the third stage is associated with hypoxia, ground-glass infiltrate with progression to acute respiratory distress syndrome (ARDS) which occurs as the virus reaches the gas exchange units of the lung infecting the peripheral and subpleural alveolar type II cells leading to apoptosis and cell death. This, in turn, will trigger a secondary pulmonary epithelial regeneration as was seen in influenza pneumonia [36], [39], [40]. The recovery will, however, require an energetic innate and acquired immune response coupled with aggressive pulmonary epithelia regeneration which is quite slow in the elderly due to their diminished immune response and reduce ability to regenerate or repair damaged epithelial cells [36].

### **Laboratory diagnosis of COVID-19**

The diagnosis of the infection is majorly by molecular methods whereby reverse-transcriptase polymerase chain reaction (RT-PCR) is the choice technique [41], [42], [43], [44], [45]; serological tests have been developed by CDC (2020) for optimization and validation of other serologic reagents manufactured for diagnostic purposes. The severity of the condition can also be evaluated by the various laboratory analyses, across the hematology, chemical pathology, histopathology, microbiology and immunology field, so as to correlate their results with the clinical manifestation in the management of the patients.

### **Specimens for diagnosis**

To ensure accurate diagnosis of the viral pneumonia infection (COVID 19), collecting good quality and the correct specimen from the patient promptly are important. According to the CDC guidelines, upper and lower respiratory specimens such as throat swab, nasal nasopharyngeal (NP) swab, sputum, and bronchial fluid are recommended [46]. In patients with more severe respiratory disease, upper respiratory specimens such as NP and oropharyngeal (OP) swab including lower respiratory specimens such as sputum (SP) and endotracheal aspirate or Broncho-alveolar lavage can be used. Previously, Wang *et al.* reported that OP swabs were used more often than NP specimen in China during the COVID-19 outbreak and that SARS-CoV-2 RNA was significantly detected in NP swabs [47]. The CDC also recommended the collection of the upper respiratory swab. Collection of an OP specimen was considered of a lower priority and when collected may be combined in a single tube as the NP swab [46]. Swab specimens are recommended to be placed and transported in a universal or viral transport medium or stored 2–8°C for up to 72 h after collection. Specimens can be stored at –70°C or below

when a delay in testing or shipping is anticipated [46]. In addition, Cheng *et al.* have recommended the collection of both upper and lower respiratory samples such as Sputum and Broncho alveolar lavage fluid [48]. SARS-CoV-2 has also been detected from urine, stool, and blood specimens, although less frequently than from respiratory specimens. Cheng *et al.* publish that 70% (n = 38) of the fecal specimen at the peak of viral (9–11 days) shedding was positive following testing. Furthermore, a case report by Tang *et al.* have recorded a positive test in an asymptomatic child who was positive for the 2019 novel coronavirus by rRT-PCR from stool specimen even 9 days after respiratory tract specimens were negative [49]. The use of nucleic acid detection of COVID-19 in fecal specimens has been appraised to be equally accurate in diagnosis [7]. Likewise, the use of serum for the detection of SARS-CoV-2 has been previously reported. Zhang *et al.* showed that serological tests can improve detection rate and recommended its usage in future epidemiological studies [50]. Rectal sourced specimens have likewise been reported positive in patients infected with SARS-CoV-2 [51].

### **Molecular diagnosis**

SARS-CoV-2 is a single-stranded positive-sense RNA virus and its entire genetic sequence was uploaded to the Global Initiative on Sharing All Influenza Data platform on January 10, 2020. The sequence data have since been helpful in designing primers and probes useful for the molecular diagnosis of SARS-CoV-2 [49], [52]. According to the WHO's guidelines, nucleic acid amplification tests (NAAT) for COVID-19 SARS-CoV-2 have been recommended. Routinely, confirmation of COVID-19 cases based on detection and analysis of the sequences of virus RNA by NAAT such as real-time rRT-PCR, followed up by nucleic acid sequencing when required. The viral genes usually targeted include the N, E, S, and RdRP genes [53]. It is recommended that RNA extraction is done avoiding contamination in a biosafety cabinet in a BioSafetyLevel-2. Heat treatment of samples before RNA extraction is not recommended [54], [55]. A major advantage in the use of molecular diagnostic strategy is its ability to detect a positive case in an asymptomatic population thus preventing the spread of the virus to close contact. A study by Mizumoto *et al.*, on the passengers (n = 3,711) on a diamond princess cruise ship tested for SARS-CoV-2 revealed an estimated asymptomatic proportion (among all infected cases) at 17.9% (95% CrI: 15.5–20.2%) [56].

### **RT-PCR**

RT-PCR is recommended as the gold standard detection method for the identification of SARS-CoV-2 virus and relies on the principle of amplifying a small



amount of viral genetic components in a sample. At present, RT-PCR test for SARS-CoV-2 has been used to test samples collected from the upper respiratory system using swabs. There are studies which have employed the use of this method in identification of SARS-CoV-2 in serum, stool, and other specimens [57], [58]. Although RT-PCR is the most widely used method for detecting SARS-CoV-2 infections, its major limitation is that it requires expensive laboratory instrumentation and reagents as well as highly skilled laboratory personnel to man such laboratories. Thus, a number of companies and laboratories around the globe are working on means of improving the efficiency and timeliness of the RT-PCR technologies and developing various other diagnostic methods [52].

Other molecular techniques in use include: Isothermal nucleic acid amplification which allows gene amplification at a constant temperature and eliminates the need for a thermal cycler [59]. This principle is used in reverse-transcription loop-mediated isothermal amplification (RT-LAMP). RT-LAMP provides an alternative and a more rapid and cost-effective testing method for SARS-CoV-2 but requires a set of four primers to target the genes and enhances the sensitivity of the test with a reverse-transcription step to allow for the detection of RNA. The amplified product can be detected using a photometric apparatus to measure the turbidity caused by magnesium pyrophosphate precipitated in the solution as a byproduct of amplification [52]. The reaction can be monitored in real time by measuring the turbidity or fluorescence using intercalating dyes since RT-LAMP diagnostic testing requires heating and visual inspection. The simplicity and sensitivity of this technique make it a promising candidate for virus detection [52], [60].

Microarrays have equally provided a rapid and high-throughput detection for SARS-CoV-2 nucleic acids. This technology relies on the use of reverse transcription to generate complementary DNA (cDNA) from viral RNA and subsequently labeling the cDNA with specific probes. In principle, the arrays are prepared either by an *in situ* synthesis of biomacromolecules on solid substrates or by spotting *ex situ* synthesized biomacromolecules on a substrate surface [61]. This is followed by loading the labeled cDNAs into the wells of microarray trays containing solid-phase oligonucleotides attached onto their surfaces. If hybridized and after washing away the unbound DNA, they remain bound, signifies the presence of virus-specific nucleic acid [41]. The use of microarray assay has been previously reported and identified as a useful tool in detecting mutations associated with SARS-CoV-2 [53], [54]. Guo *et al.* detected and characterized viral strains with 100% accuracy and thus recommended this method for the detection and epidemiological surveillance for SARS-CoV-2 [62], [63]. At present, more studies are needed to further substantiate and to evaluate the sensitivity of this method in SARS-CoV-2 diagnosis especially in Africa.

### **Serological and Immunological assays**

Serologic assays have important public health applications in the current COVID-19 response. RT-PCR-based viral RNA identification has been used globally and appraised as the gold standard in diagnosis of COVID-19 but has inability to monitor the progress of the disease stages and cannot be applied to the identification of past infection and immunity [52]. It is believed that serologic testing can aid the understanding of disease patterns and transmission as well as improve data collection from serologic surveys among different ethnic groups and populations [64].

Serologic testing involves analysis of blood serum or plasma and other biological fluids. In principle, it involves recognition of antibodies, which are specific proteins produced in response to infections. This screening method plays an important role in epidemiology and vaccine development. It also provides an assessment of both short-term (days to weeks) and long-term (years) trails of antibody response in the tested population. Immunoglobulin M (IgM) first becomes detectable in serum after few days and lasts for some weeks on infection. This is usually followed by a switch to immunoglobulin G (IgG). Thus, IgM may be used as an indicator for early stage of infection and IgG is indicating a current or prior infection. IgG may also be used to suggest the presence of post-infection immunity. In the recent COVID-19 pandemic, serologic tests have been identified to have huge potential for the epidemiology of COVID-19 [64].

However, the United States' CDC has cautioned on the direct adoption of serologic testing for SARS-CoV-2 as more studies are needed to comprehend if the antibodies produced from SARS-CoV-2 infection will confer immunity from a future infection. CDC scientists are conducting studies to better understand the level of antibodies needed for protection, the duration of such protection, and the factors associated with whether a person develops a protective antibody response [64]. The U.S. Food and Drug Administration (FDA) recommended (revised policy) that the use of serologic tests and reagents intended for antibody detection to SARS-CoV-2 to identify people who may have been exposed to the SARS-CoV-2 virus or who have recovered from the COVID-19 infection be subjected to validation and approval, it was also emphasized that serological (antibody) tests should not be used as the sole basis to diagnose COVID-19 [65].

The serologic techniques in use include the enzyme-linked immunosorbent assay (ELISA), immunochromatographic (ICG) lateral flow assay, neutralization bioassay, and specific chemosensors. They vary in speed, multiplexing, automation, as well as limitations such as requirements for trained personnel and availability of dedicated laboratories. The FDA had earlier granted emergency use authorization status to the first serology test, qSARS-CoV-2 IgG/IgM Rapid Test, manufactured by Cellex Inc., on April 1, 2020 [65].

A study by Pan *et al.* demonstrated the serologic ability of ICG strips in identifying SARS-CoV-2 infection to be sensitive and consistent and thus considered it as an excellent complementary approach in clinical application [66]. In another study, Haveri *et al.* assessed the presence of neutralizing antibody response along with specific IgM and IgG targeting nucleocapsid and spike proteins and recommended this technique has a candidate test which can enhance better understanding of SARS-CoV-2 infection. However, monitoring of the binding antibodies is suggested to be a more sensitive method than measuring functional neutralizing antibodies for serological detection of hCoV infections [67].

### Hematological features and other laboratory findings

The hematological analysis reported by various researchers include the total leukocyte count, lymphocyte, eosinophils, monocyte, platelet counts, D-dimer, procalcitonin (PCT), serum amyloid A (SAA), erythrocyte sedimentation rate (ESR) and they are categorized based on the concentration of parameters, severity of disease (severe and non-severe conditions), 5 and 4-days post-hospital presentation, and complication conditions as represented in Table 1 obtained from the studies by Zhang *et al.* and Bingwen *et al.* [1], [68]. Zhang *et al.* reported that the total leukocyte count, D-dimer, C-reactive protein (CRP) ( $p < 0.001$ ), and SAA were increased in patients with severe condition than those whose conditions were not severe while decreased value was observed in lymphocyte, eosinophils, and PCT in severe conditions with observable lymphopenia and eosinopenia at 5 days post-clinical presentation. The population distribution demonstrated 12.3%, 43.2%, 34.7%, and 90.2 % of the COVID-19 positive population had increased leukocyte, D-dimer, PCT, and SAA, respectively, while 19.6 %, 75.4%, and 52.96 % had decreased leukocyte, lymphocyte, and eosinophil counts, respectively [1]. In addition, corroborating work by Bingwen *et al.* reported similar white cell results along with moderate platelet count and hemoglobin concentration [68]. Some other studies that relate COVID-19 infection with thrombosis also observed increased level of D-dimer along with increase in some other coagulation markers such as fibrin, fibrin degradation products (FDP) with minimal change in prothrombin time, activated partial thromboplastin time, and platelet count which are pointers of thrombosis as reviewed by Connors *et al.* and Robbin *et al.* [32], [69]. In addition, the assessment of 11 patients in the intensive care unit in Italy display reduced mean antithrombin concentrations with slightly low protein S free antigen along with an increased mean vWF and vWF-ristocetin cofactor activity [70]. These observations coupled with other severe thrombotic developments led the ISTH developing an interim guidance on recognition and management of coagulopathy in COVID-19 whereby D-dimer, prothrombin time, platelet count, and fibrinogen (if fibrinogen measurement is feasible) are to be measured

**Table 1: Distribution of hematological parameters across various severity conditions and post-presentation days as described by Zhang et al. (2020) [1] and Bingwen et al. (2020)**

Parameters	Condition/n-138 (Zhang et al., 2020) [1]		p-value	Condition at 5 days post-presentation/n-138 (Zhan et al., 2020)		p-value	Condition/n-67 (Bingwen et al., 2020)		p-value	Condition at 4 days post presentation/n-67 (Bingwen et al., 2020)		p-value
	Non-Severe	Severe		Non-severe	Severe		Non-severe	Severe		Non-severe	Severe	
Hb (g/dl)	-	-	-	-	-	-	-	-	-	-	-	-
Total leukocyte ( $\times 10^9/L$ )	4.5 (3.5-5.9)	5.3 (4.0-9.0)	0.014	-	-	-	14.2 (12.9-15.2)	13.2 (12.5-14)	0.70	13.6 (12.7-15.1)	11.1 (10.2-11.9)	<0.001
Lymphocyte ( $\times 10^9/L$ )	0.8 (0.6-1.2)	0.7 (0.5-1.0)	0.048	1.1 (0.7-1.6)	0.7 (0.4-1.1)	<0.001	4.7 (4.0-5.8)	5.1 (3.5-8.2)	0.87	1.2 (0.8-1.6)	0.4 (0.3-0.5)	<0.001
Monocyte ( $\times 10^9/L$ )	-	-	-	-	-	-	1.3 (0.9-1.7)	0.5 (0.48-0.8)	<0.001	0.4 (0.3-0.5)	0.2 (0.19-0.23)	<0.001
Eosinophil ( $\times 10^9/L$ )	0.02 (0.008-0.05)	0.01 (0.0-0.06)	0.451	0.04 (0.01-0.08)	0.01 (0.0-0.09)	0.157	0.5 (0.4-0.6)	0.3 (0.2-0.5)	0.12	0.4 (0.3-0.5)	-	-
Neutrophils ( $\times 10^9/L$ )	-	-	-	-	-	-	2.6 (2.1-3.8)	4.2 (2.1-6.9)	0.17	3.5 (2.6-4.4)	11.6 (9.3-13.8)	<0.001
Platelets ( $\times 10^9/L$ )	0.2 (0.1-0.3)	0.4 (0.2-2.4)	<0.001	-	-	-	201 (157-263)	217 (154-301)	0.81	192 (150-261)	154 (131-216)	<0.001
D-dimer ( $\mu g/ml$ )	28.7 (9.5-52.1)	47.6 (20.6-87.1)	<0.001	-	-	-	-	-	-	-	-	-
CRP (mg/ml)	0.05 (0.03-0.1)	0.1 (0.06-0.3)	<0.001	-	-	-	-	-	-	-	-	-
PCT (ng/ml)	91.5 (24.9-163.2)	108.4 (64.1-161.6)	0.600	-	-	-	-	-	-	-	-	-
SAA (mg/L)	-	-	-	-	-	-	-	-	-	-	-	-

p-value denotes comparison between the severe and non-severe groups across each study [1], [59].

in patients presenting with COVID-19. The patients with abnormal results are to be admitted and subsequent treatment (heparin or blood product transfusion) based on the severity of results obtained [71].

Furthermore, another study by Shuchang *et al.* on the CT features of COVID-19 infected persons with pneumonia in some 62 patients in Wuhan, China, observed that the routine blood analyses conducted on 30 of the patients displayed 6 (20.0%), 24 (80.0%), and 15 (50.0%) with leucopenia, lymphocytopenia, and decreased percentage of lymphocytes, respectively [72]. The ESR and high-sensitivity CRP level assessed yielded 66.7% and 100% elevated values for both parameters, respectively, thus are corroborating the report of the study by Zhang *et al.* [1]. A usual or diminished total white blood cell count along with reduced lymphocyte count can be demonstrated in the early phase of the infection as researched by Cascella *et al.* [73]. Furthermore, their article reported that lymphopenia is visibly negative prognostic factor for COVID-19 infection with amplified values of CRP and standard procalcitonin value. In severe condition, it was reported that D-dimer value is augmented, blood lymphocytes declined persistently, and analytical variations of multiple organ imbalance evident by high amylase as well as disordered coagulation activities [73].

In addition, leukopenia, lymphocytopenia, and eosinophil cytopenia were observed to be recurrent in COVID-19 induced pneumonia more than those in non-COVID-19 induced pneumonia [74]. Furthermore, a marked reduction in CD4 and CD8 lymphocytes series was noted at the onset phase of the disease by Wang *et al.* [8] with patients in the critical care facility displaying higher levels of interleukin (IL) 2, IL-10, IL-7, monocyte chemotactic protein 1 (MCP1), granulocyte colony-stimulating factor (GCSF), interferon gamma-induced protein 10 (IP10), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and macrophage inflammatory protein alpha along with other abnormal coagulation, and cell count parameters described by some other researchers [7], [75]. The lung injury presentation in this infection has been strongly analyzed by emerging evidences to result from the excessive release of pro-inflammatory markers such as IL-1, IL-6, and TNF- $\alpha$  and interferon, a situation called cytokine storm [76]. This is because their secretion results in influx of immune cells into the site of infection producing destructive effects on tissues damaging cell interaction, multi-organ failure, and eventual death [77]. This is ascertained by the further analysis of cytokines in infected patients with the observation that they are increased in patients and even more elevated in severe cases [7], [78], [79], [80], [81], [82] as well as in children from ages 2 months to 15 years [83].

In addition, infected individuals with blood Group A were studied to be significantly most-at-risk for contracting COVID-19 compared with non-A blood groups while blood Group O has a appreciably lower risk for the infection compared with non-O blood groups

as reported by some researchers in Wuhan, China [19]. Although, there are still some categorical questions concerning the study with regard to the distribution of blood groups in China populace, ethnicity, and statistical inferences.

### **Histopathological features of COVID-19**

The macroscopic view of COVID-19 is observed more to be in the chest and can also include pleurisy, lung consolidation, pulmonary edema, and pericarditis. The weight of the lung may increase above normal. It can also be noted that a secondary infection can be super imposed on the viral infection which can lead to purulent inflammation that is more of a typical bacterial infection [84]. A recent article explained the early pathological features in COVID-19 in two patients who had gone through surgical resections for lung adenocarcinoma, but it was later discovered during the procedure of the operation that the two patients had COVID-19 [11]. The findings obtained were not specific and these include edema, pneumocyte hyperplasia, focal inflammation, and multinucleated giant cell formation, while hyaline membranes were not observed. These patients did not show any symptoms of COVID-19 as at the time of the operation; these are likely to reflect only in the early changes of acute lung injury in the infection [11].

Furthermore, a reported case of biopsy samples from 50 years deceased of COVID-19 in Wuhan city, China, was harvested from the liver, lungs, and heart organs of the patient. Histomorphological examination revealed bilateral diffuse alveolar damage with cellular fibromyxoid exudates [85]. The right lung revealed evident of desquamation of pneumocytes and hyaline membrane formation, which indicates an ARDS. The left lung showed pulmonary edema with hyaline membrane formation, which suggests of early-phase ARDS [85]. Both the right and left lungs also revealed interstitial mononuclear inflammatory infiltrates, which are dominated by lymphocytes. Multinucleated syncytial cells with atypical enlarged pneumocytes, characterized by large nuclei, amphophilic granular cytoplasm, and prominent nucleoli were observed in the intra-alveolar spaces, which showed viral cytopathic like changes [85]. There were no obvious intracytoplasmic or intranuclear viral inclusions were seen. In addition, the liver biopsy specimen of the same patient with COVID-19 revealed moderate microvesicular steatosis, mild lobular, and portal activity which indicates that the injury might have been caused by either SARS-CoV-2 infection or drug induced liver injury. There was no other substantial damage observed in the heart tissue except a few interstitial mononuclear inflammatory infiltrates [85]. According to Sufang *et al.*, 2020, who also carried out postmortem fine-needle core biopsies on some selected organs (liver, heart, and lungs) on four patients who also died of COVID-19 pneumonia, each of these patients had one or two underlying diseases, which include



immunocompromised status (chronic lymphocytic leukemia and renal transplantation) or some other conditions, which are liver cirrhosis, diabetes, and high blood pressure [86]. The time frame from the onset of the disease to death for the patients ranged from 15 to 52 days. The main features observed in their histology were in the epithelial tissues, formation of hyaline membrane, and hyperplasia of type II pneumocytes, all the components of diffuse alveolar damage. Fibroblast proliferation with extracellular matrix and fibrin forming clusters in airspaces is evident with an abundant intra-alveolar neutrophilic infiltration, consistent with superimposed bacterial bronchopneumonia in one of the patients. The liver showed a mild lobular infiltration with small lymphocytes and centrilobular sinusoidal dilation and a patchy necrosis was also observed [86].

The heart showed only focal mild fibrosis and mild myocardial hypertrophy. The postmortem examination showed an advanced diffused alveolar damage as well as superimposed bacterial pneumonia in some of the patients. The histomorphological changes observed in the heart and liver were most likely to be secondary or caused as a result of the effects of the underlying disease [86].

## Complications of COVID-19 Infection

### *Acute respiratory syndrome*

The presence of ACE2 on the cells of the heart, kidney, and lungs encourages the SARS viruses infection [87] and about 42% of COVID-19 patients do develop ARDS [88]. Diabetes mellitus is also a factor associated with the development of ARDS [78]. Other diseases that can cause complications in COVID-19 patients include hypertension, cardiovascular disease, and chronic kidney disease [88], [89]. Medical laboratory findings associated with the development of ARDS include lymphopenia, neutrophilia, elevated CRP, elevated blood urea nitrogen, prolonged prothrombin time, elevated d-dimer, and elevated lactate dehydrogenase (LDH) [88], [89].

About 36% of ARDS cases are mild, 45% cases are moderate while 18.9% cases are severe [17]. Mortality increases with the severity of the disease. Patients above 65 years of age do have worse degree of ARDS and they also have higher mortality likelihood [89]. Medical laboratory markers that can predict the mortality of COVID-19 ARDS patients include low albumin, elevated blood urea nitrogen, and elevated LDH [88], [89].

### *Myocardial injury*

Most commonly causes of COVID-19 related deaths are usually associated with the hearts and

lungs [90]. There are two main theories explaining the mechanism by which myocardial injury occurs with COVID-19. The first theory explains that the heart has similar ACE2 levels as that of the lungs [77], which allows viral entry into the myocardial cells [91]. The second theory involves a cytokine storm causing myocardial injury [91]. Myocardial injury includes heart failure, myocarditis, acute coronary syndrome, hypotension, or shock and sepsis [92]. To have a definitive characterization of the injury, endocardial biopsy is possibly required and magnetic resonance is required [92].

Arrhythmias have been observed to arise with severe COVID-19 cases [93], [94]. Malignant arrhythmias, including ventricular tachycardia and fibrillation, always occur at a rate of 6% and can be elevated more frequently in patients with elevated troponin levels (17.3% of patients with elevated troponin) [85]. Heart failure is commonly observed in severe cases of COVID-19, irrespective of previous cardiac history [85]. This presents with elevated levels of N-terminal pro B-type natriuretic peptide (NT pro-BNP) and troponin levels, mostly in severe cases [49]. Some studies have also revealed that pulmonary hypertension which causes right heart failure can also contribute to these cases.

Elevated high sensitivity troponin (HS-troponin) and creatinine kinase-myocardial band (CK-MB) levels can also self-sufficiently predict severe COVID-19 cases [88], [92], [93], [94], [95]. A recent meta-analysis revealed that troponin is more elevated in severe cases [96]. Cytokines do not predict severity [88]. It has been revealed that patients with elevated HS-troponin ( $\geq 28$  ng/L) and CK-MB are suspected to have myocarditis or heart failure [7], [88].

### *Acute kidney injury*

Acute kidney injury presents with elevated urea and cystatin-C levels in severe COVID-19 infection [89]. Concerning the cause of acute kidney injury, there are two hypotheses to it. One of the hypotheses is from kidney able to harbor more ACE-2 levels than the lung or heart, most especially in the proximal convoluted tubules. However, COVID-19 RNA is not encountered in the urine. The second theory has to do with the injury incurred through a cytokine storm [96]. Patients may need to do continuous renal replacement therapy (CRRT) due to the severity of kidney injury. Speculation exists concerning CRRT potentially serving as a way of removing large cytokine levels from the system, regardless of kidney injury [96].

The prognosis predictors of COVID-19 infection include cardiovascular disease, hypertension, cerebrovascular disease, and chronic kidney disease [48] which can all result in severe conditions. In patients with cardiovascular disease, they present with a 10.5% coronary flow reserve (CFR); other diseases

that can also present with a high CFR include chronic lung diseases (6.3%); cancer (5.6%); hypertension (6.0%); and diabetes.

## Conclusion

The diagnostic analysis of this new infection is majorly by NAAT such as real time RT-PCR followed by nucleic acid sequencing. Although some serologic and immunologic assays such as ELISA and ICG lateral flow assay have been developed which detects the IgM and IgG antibodies, there are still lots of cautious remarks on the direct adoption of these tests due to the need for more studies to determine the immunologic capabilities and strength of the antibodies in prevention of future infection. Based on severity, the laboratory features of COVID-19 infection include lymphopenia, eosinopenia, reduced procalcitonin along with leucocytosis, increased D-dimer, CRP, SAA, fibrin, FDP with mild to moderate increase in PT, and APTT while the major pathologic presentations present with features of acute respiratory syndrome, myocardial injury, and acute kidney injury. Furthermore, some inflammatory markers such as IL-2, IL-6, IL-7, IL-10, GCSF, MCP10, IP10, and TNF- $\alpha$  are apparently increased in COVID-19 infection signaling cytokine storm in the course of the infection and associated SIC. Therefore, as much as there are some established pathologies, diagnosis, and laboratory features of COVID-19 by clinical presentation and hospitalization, more studies are required to identify the capabilities, characteristics and tendencies of this novel virus as well as the most efficient prevention and treatment.

## Author's Contributions

All authors contributed responsibly to this manuscript, have accepted accountability for the entire content, and approved its submission.

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