International Journal of Medical and Health Research
ISSN: 2454-9142
Received: 20-08-2020; Accepted: 05-09-2020; Published: 21-09-2020
www.medicalsciencejournal.com4
Volume 6; Issue 9; 2020; Page No. 77-82

A brief overview on potential prognostic biomarkers in diagnosis of SARS-CoV-2 infection

Mir Naiman Ali[1*], Hajera Tabassum[2], Roua Alsubki[3]
[1] Head, Microbiology Section, Riyadh Municipality Central Area Labs, Riyadh, Saudi Arabia
[2] Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia
[3] Chair of Medical and Molecular Genetics, Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia

Abstract
The world is under pandemic stress due to increased mortality rate owing to the outbreak of novel corona virus termed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 has recently emerged from Wuhan city of China on 31st December 2019. As of 31 August, 2020 a total of 25118689 confirmed cases and 844312 deaths were reported globally indicating urgent necessity to contain the spread of this contagious virus (WHO COVID-19 data). Moreover, patients infected with SARS-COV2 are at risk of adverse complications including cardiovascular diseases - myocardial infarction, liver injury, multiorgan failure and death. In view of the staggering mortality rates reported, it is imperative to diagnose patients with COVID-19 and identify biomarkers that assist in identification of high risk patients. Subsequently, the present review was undertaken to identify prognostic markers associated with morbidity and mortality in COVID-19. Search of the evidences across published meta-analyses or systematic reviews currently investigating on the different types of biomarkers – Blood cell count, inflammatory markers –CRP and cytokines, coagulation markers-D-dimers, cardiac and renal markers associated with COVID severity was accomplished. The outcome of the study provides an insight of the potential prognostic markers that can be adopted as POCT (Point of care testing) in diagnosis of SARS-CoV 2 infection and assist physicians in stratification of the diseased, triage and treatment of patients with COVID-19 disease.

Keywords: Coronavirus, COVID-19, biomarkers

Introduction
The planet is currently facing a pandemic stress as a result of the spread of the novel Corona virus (Co V), named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). World Health Organization (WHO) termed the disease caused by SARS-CoV-2 as COVID-19. SARS-CoV-2 has recently emerged from Wuhan city of China on 31st December 2019 with a total of 25118689 confirmed cases and 844312 reported deaths globally and 315772 confirmed cases in Kingdom of Saudi Arabia (as of 31st August 2020) [WHO COVID-19 data & WHO situation report 125 Kingdom of Saudi Arabia] [1, 2]. The patients infected with COVID-19 display symptoms like cough, high fever, fatigue, septicemia, adverse acid-base metabolism, malfunctioning of respiratory system leading to life threatening consequences and in some cases acute respiratory distress syndrome (ARDS), acute cardiac injury or secondary infection [3, 6]. Corona viruses are ss RNA (+ve sense), enveloped, spherical, with prominent spiked S glycoprotein on its surface. The nucleocapsid is enclosed in an envelope and matrix [7, 9]. It has a diameter of 50–200 nm and possesses spikes on its surface (up to 20 nm in length) that provide it the crown-like appearance, a characteristic of coronaviruses (CoVs) [10]. Timely and accurate COVID-19 laboratory testing is an essential part of the management of COVID-19 for slowing down the pandemic and supporting decisions on infection control strategies. With cases of COVID-19 disease, rising at an unprecedented rate, there is urgent need for development of diagnostic tests for rapid identification of COVID 19 infected people. It is vital to diagnose affected patients as quickly as possible for early containment and treatment and also for immediate identification of patients into risk groups –mild and severe. Therefore, it is imperative to identify biomarkers that could effectively functions as diagnostic tools in COVID-19 testing.

Currently, real time polymerase chain reaction (RT-PCR) is the primary diagnostic tool to detect cases of SARS-CoV-2 infection from nasal and pharyngeal swabs and bronchoalveolar lavage (BAL) fluids. In addition, computed tomography (CT) imaging and some hematology parameters supplement the diagnosis**.** But, the real-time PCR tests have long turn-around times (2 to 3 hours) to generate results and are complicated in operation. Furthermore, molecular diagnostic tools require expensive equipment. In order to minimize the cost and time in diagnosis, novel biomarkers whose levels varies with disease severity must be recognized**.** Identification of novel biomarkers relies on the clear understanding of the etiology behind SARS-CoV-2 infection. While the underlying pulmonary pathophysiology remains incompletely unresolved, presumably severe COVID-19 infection is found associated with a marked alveolar inflammatory cell infiltration, and cytokine storm response[11] causing vascular endothelial damage, activation of the coagulation system, and inhibiting the fibrinolytic and anticoagulating systems. Disseminated intravascular coagulation (DIC) can result due to excessive thrombosis in the microvascular system, microcirculatory disorder and consequently to serious multiple organ dysfunction syndrome. Blood coagulation as a fatality threat in COVID 19 patients was evidenced by Tang *et al*., 2020 when the levels of coagulation markers (thrombin, fibrin related

markers etc) increased markedly in these subjects. Moreover, emerging data suggest that severe COVID-19 is also associated with a significant increased risk for developing deep vein thrombosis and pulmonary embolism [12, 13]. Strikingly, COVID-19 patients are found more succeptible to cardiovascular diseases (CVD) with blood coagulation identified as primary etiological process responsible for multiple organ failure. Henceforth, biomarkers involved in coagulation and cardiovascular risk also must be identified that could be useful in diagnosis of COVID-19 patients.

In view of the above, a comprehensive review was performed searching available data on the biomarkers in COVID 19 detection on PubMed, SCOPUS, and Google Scholar. This article aims to explore the role of different biomarkers in the pathogenesis of COVID-19 disease and provide clear insights into the usability of these biomarkers in predicting the severity of the disease and assist the clinicians to adapt therapeutic strategies following the diagnosis. Various potential hematological, biochemical, immunological and coagulation markers that are reviewed here includes C-reactive protein (CRP), IL-6, white cell count (WCC), lactate dehydrogenase (LDH), D-dimers, cardiac troponin and renal markers.

Method

Search strategy: A comprehensive literature search was done on PubMed, SCOPUS and Google Scholar to identify articles discussing biomarkers in this review and its clinical implications on COVID-19. Search was performed by using following keywords- COVID-19, SARS-COV-2, antibody detection, cytokines, white cell count, biomarkers, biochemical markers, C-reactive protein, neutrophil count, lymphocyte count, coagulation markers, D-dimer, platelet count, cardiac troponin, renal biomarkers.

Data extraction: All articles were subjected for screening and validation by two authors. Studies correlating biomarker with COVID-19 severity were included.

Statistical analysis: It was not possible to conduct an appropriate meta-analysis because there were not enough research data among the studies on this subject.

Results

Table 1 summarizes the studies reporting data on biomarkers varying with COVID-19 severity and detection

1. *Antibodies*

Preliminary screening of IgG /IgM antibodies as point of care (POC) test is an indicator of infection, is quick (with results in 10 minutes) and would be better approach in early diagnosis of COVID-19. As known, IgM provides the first line of defense against viral infections preceding the synthesis of high affinity immunoglobulins G (IgG) for long-term immunity and immunological memory. Hence, the detection of IgM in the serum is indicative of a recent exposure to the virus, while the detection of IgG suggests that the exposure occurred several days before. Guo *et al*., 2020 while investigating the humoral response against SARS-CoV-2, analyzed IgA, IgM, and IgG response, on the recombinant viral nucleocapsid protein (NP) to determine the diagnostic value of IgM[14]. Around 90.4% and the 93.3% of 208 patients harbored plasma IgM and IgA, respectively, and the 77.9% of plasma samples were positive for IgG. Similar results were evidenced by Long *et al*., 2020 on a cohort investigating 285 patients with COVID-19[15]. 100% of patients were tested positive for antiviral immunoglobulin-G (IgG) and within 19 days seroconversion for IgG and IgM occurred simultaneously with flattening of both IgG and IgM titers within 6 days after seroconversion.

2. *Blood cell count*

Levels of hemoglobin, WBC, lymphocytes, neutrophil, eosinophils, platelets and neutrophil–lymphocyte ratio (NLR), have been researched to find the prognostic value in onset and progression of COVID-19 disease. In a study by Yang *et al*., 2020, around 80% of critically ill adult COVID-19 patients develop lymphopenia [16]. Parallely, Chen *et al*., 2020 reported a rate of 25% in these subjects reflecting the association between lymphopenia and disease severity [17]. In a meta-analysis, elevated levels of WBC with lowered lymphocytes and platelets was reported in severe and fatal cases in cohort involving 3377 COVID positive cases [18]. In accordance, in a retrospective study by Qin *et al*., 2020 investigating 450 COVID positive cohort found reduced lymphocytes, monocytes, eosinophils, basophils and higher NLR in severe cases compared to mild ones [19]. Neutrophils were predominantly driving this increase as the severe set (4.3 vs 3.2 × 10^9/L; P<0.001). Both groups experienced an increase in leucocytes with the severe group having a significantly greater rise. However in severe disease, WBCs show lymphocytopenia, both $CD4^+$ and $CD8^+$ cells, as well as a decrease in monocytes and eosinophils, and a clear increase in neutrophils and NLR. These simple parameters can be used for early diagnosis and identification of critically ill patients.

Although most of the studies are on Chinese subjects, further in a national multi-center and retrospective cross-sectional study in Saudi Arabia investigating on the clinical characteristics, and outcomes of COVID-19 cases across all the regions of Saudi Arabia found pneumonia, the most frequent manifestation concomitant to Acute Respiratory Distress Syndrome (ARDS) [20]. Besides these symptoms leukopenia, leukocytosis, and lymphopenia, with altered white blood count in severe COVID 19 patients with predictive value has been reported [21]. Though, the data on eosinopenia provides evidence as diagnostic marker, it cannot be used as prognostic marker as the WBC levels alter in bacterial /other viral infections including glucocorticoid therapy. Nevertheless, a number of studies have evidenced reduced platelet count in critically ill COVID-19 patients, and non survivors indicating its usability as a potential biomarker for detection of SARS-CoV-2 infection. In Meta-analysis by Lippi *et al*., 2020 studying 1799 patients revealed decreased levels of platelet count among patients with severe COVID-19 infections [22].

Besides, levels of T cells are found varying with severity in SARS-CoV-2 infection. The mean lymphocyte counts were below normal among COVID-19 cases [23]. Lowered levels of T cells count, an increase in naive helper T cells and a decrease in memory helper T cells was recorded in patients severely affected by COVID-19[24]. The total number of natural Killer cells, T cells, and B cells was decreased markedly in patients with SARS-CoV-2 infection

3. *Biochemical biomarkers*

Biochemical profile of severe /non-survivors with mild COVID-19 disease cases yielded informatory results. Levels

of a number of biochemical markers were found altering with disease severity. Ruan *et al*., 2020 whilst investigating the clinical predictors of mortality due to COVID-19 in two groups– death and discharge from COVID-19, observed that the laboratory results exhibited significant differences in white blood cell counts, absolute values of lymphocytes, platelets, albumin, total bilirubin, blood urea nitrogen, blood creatinine, myoglobin, cardiac troponin, C-reactive protein (CRP) and interleukin-6 (IL-6) between the two groups [25]. These observations were in line with reports of Yang *et al*., 2020 and Zhou *et al*., 2020 [26, 27].

Liver function has also been identified as an important predictor for COVID-19 mortality. In a retrospective analysis involving a COVID-19 cohort-derived data from 5771 patients, Lei *et al*., 2020 reported AST (aspartate aminotransferase) to be strongly associated with mortality risk compared to other parameters, reflecting liver injury[28]. Parallely, Chen *et al*., 2020 reported increased /elevated levels of ALT (alanine aminotransferase), AST, creatinine, CK (creatine kinase), LDH (Lactate dehydrogenases), cardiac troponin I, N-terminal pro-brain natriuretic peptide, and D-dimer in non-survivors compared to recovered patients in a cohort of 799 patients surveying 113 non-survivors and 161 recovered [29].

Role of Lactate dehydrogenases (LDH) the enzyme in glucose metabolism catalyzing conversion of pyruvate to lactate has also yielded affirmative results. Presumbling elevated levels of this enzyme could be a consequence of cell necrosis owing to the SARS-CoV-2 infection. Ferrari *et al*., 2020 found significantly higher levels of LDH in ICU patients than non-ICU patients (248 U/L vs 151 U/L, p=0.002) [30]. Since high levels of LDH continued in the ICU patients number of days post-admission (160 U/L vs 218 U/L, p=0.002), LDH may be a predictive biomarker of severe disease. However, the one centre study may be liable to selection bias which could potentially reduce its validity.

Similar trends has been observed for renal markers. Studies have demonstrated significantly higher levels of renal biomarkers such as serum urea, creatinine and markers of glomerular filtration rate in severe cases. Cheng *et al*., 2020 observed that raised levels of serum creatinine levels on admission correlated with severity due to significant abnormalities in the coagulation pathway [31]. Thus suggesting that the renal abnormalities on admission may indicate higher risks of deterioration, ensuring appropriate triaging of COVID patients.

4. *Inflammatory biomarkers and their diagnostic potential in SARS-CoV-2.*

CRP (C-reactive protein) as an indicator of inflammation is evidenced in many studies. Nevertheless, its association with COVID-19 severity yielded informative findings. In a retrospective single-centre study by Qin *et al*., 2020 in Wuhan, China significantly higher levels of CRP in severe cohort compared to the non-severe cohort (57.9 mg/L vs 33.2 mg/L) had been reported suggesting the prognostic value of CRP in COVID-19 [19]. Intriguingly, Tan *et al*., 2020 evaluation of the CRP as biomarker to predict the progression of COVID-19 infection found significant association between C-reactive protein and computed tomography (CT) in predicting severe COVID-19 at early stages [32]. The main contributing factor which could be dangerous and fatal in SARS-COV 2 is the generation of cytokine storm. Lymphopenia and interstitial pneumonia augmented with high levels of pro-inflammatory cytokines including IL-2, IL-6, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1α and TNFα can ensue in severe cases. Consequently, the enormous release of cytokines creates the so-called "cytokine storm" which, in turn, can induce acute respiratory distress syndrome (ARDS), respiratory failure, organ failure and possibly the patient's death. A number of cytokines have been evaluated for their role as potent immunological biomarkers during infection by CoVs, as immunopathology has been suggested as a primary driver of morbidity and mortality with COVID-19. Several cytokines and other immunologic parameters have been correlated with COVID-19 severity. Most notably in a study by Chen *et al*., 2020, elevated IL-6 levels were identified in hospitalized and critically ill patients, and were related with respiratory failure, and poor prognosis [33]. Furthermore, Gong *et al*., 2020 and Zhou *et al*., 2020 observed association between raised levels of IL-2R, IL-8, IL-10, and GM-CSF with disease severity, yet further studies with larger cohorts of patients are required to designate predictive control [34, 35]. Results of few cytokines studied were inconsistent. Contradictory results were reported in the role of IL-1b and IL-4 results [36]. Yang *et al*., 2020 documented that although elevated cytokine concentrations have been widely described in COVID-19 patients, the vast majority (including IL-6, IL-10, IL-18, CTACK, and IFN-g) do not seem to have prognostic value, because they do not always differentiate moderate cases from severe cases [37]. This stratification was possible with IP-10, MCP-3, and IL-1ra. Nevertheless, the study by Herold *et al*., 2020 yielded satisfactory results. The levels of IL-6 at first assessment was suggestive to predict respiratory failure [38]. Parallely, Zhou *et al*., demonstrated that IL-6 increases fairly late during the diseases course and subsequently has its prognostic value at earlier stages [39].

Furthermore, role of procalcitonin (PCT) synthesized by extra-thyroid tissue has also been researched. A rise of PCT over 100 ng/ml appeared to raise during severe infection (bacterial, parasitic, and fungal) with systemic manifestations [40]. Although its biological action is largely unknown, the sequence homologies between PCT and other human cytokines, such as TNF-a family, IL-6, etc., support the hypothesis that PCT is a mediator of inflammation [41]. As for COVID-19 patients, more severe cases showed a more marked increase of PCT compared with non-severe cases [42, 43]. A slight increase (much less than 0.5 ng/mL) in PCT levels is an important indicator to distinguish between SARS-CoV-2-positive and SARSCoV-2-negative patients and increased PCT values have been associated with a nearly fivefold higher risk of severe SARS-CoV-2 infection (odds ratio (OR): 4.76; 95% CI: 2.74–8.29) [44]. Although initial PCT value may be helpful in the determination of illness severity, it may not always be a reliable prognostic indicator. As PCT values may be influenced by preexisting comorbid conditions, such as chronic kidney disease and congestive heart failure, baseline values may be high. However, PCT can provide invaluable information if considered within the clinical context.

Recently, role of Angiotensin (Ang) was confirmed with involvement of ACE 2 in host cell entry by Chen *et al*., 2020 [45]. In the renin–angiotensin system (RAS), ACE2 functions as a regulator of modulating endogenous levels of Ang I and Ang II [46]. ACE2 counteracts the RAS cascade by providing inhibitory control over Ang II. Presumbly ACE2

directly catalyzes Ang I and Ang II, exhausting their levels. The mechanism of downregulation of ACE 2, during SARS-COV-2 infection was evidenced by Hanff *et al*., 2020[47].The enhanced production of Ang II was found concomitant with onset of ARDS and myocarditis. Supportingly, Liu *et al*., 2020 reported an increased levels of plasma Ang II level in SARS-CoV-2 infected patients linearly associated to viral load and lung injury [48].

The associated risk of CVDs in COVID-19 disease is subject of immediate concern. Researchers have enhanced their investigation on evaluation of cardiac biomarkers that acts as prognostic markers with greater risk of CVD in COVID-19 cases. Yang *et al*., 2020 in a meta-analysis involving 8 studies with a total of 46,248 COVID-19 patients in total, reported CVD as the third most prevalent comorbidity in COVID-19 patients, and patients with severe COVID-19 symptoms had a higher risk of CVD [49]. Furthermore, assessment of cardiac biomarkers in patients with COVID-19 was systematically reviewed by Mahajan *et al*., 2020 [50]. COVID-19 was associated with acute cardiac injury in around 7-28% of patients, significantly increasing its associated complications and mortality. Patients with underlying cardiovascular disease are more prone to develop acute cardiac injury as a result of COVID-19. Parallely, Huang *et al*, 2020 demonstrated the predictive potential of troponin proteins for severe morbidity in COVID-19 patients [51]. An elevated cTnI levels (>28 ph/mL) in 5 out of 41 (12%) COVID-19 patients were reported. All 5 then developed acute myocardial injury, and 4 out of the 5 were admitted into an ICU—this allows the conceptualisation of cTnI as a prognostic tool in other diseases such as COVID-19. Nevertheless, Shi *et al*., 2020 also identified that about 19.7% of COVID-19 patients (82/416) with myocardial injury, were diagnosed by significantly raised serum cTnI levels [52]. Furthermore, an increased mortality rate of 51.2% in patients with elevated cTnI levels compared to a 4.5% mortality rate in those with normal cTnI levels with no myocardial injury, suggested the interplay of myocardial injury in COVID-19 patients.

In addition, the possible role of cTnT in COVID-19 prognosis was also demonstrated by Guo *et al*.,2020 with the elevation of cTnT levels in 27.8% ;52 out of 187 hospitalized COVID-19 patients, all of whom developed myocardial injury [53]. A staggering mortality rate of 59.6% compared to 8.9% in those patients with normal serum cTnT levels was observed. Whilst COVID-19 patients with raised cTnT levels and established CVD had an alarming mortality rate of 69.4%, those with raised serum cTnT levels but no history of CVD still had a relatively high mortality rate of 37.5%. This indicated the prognostic value of detecting elevated cTnT levels in all COVID-19 patients, irrespective of the presence of underlying CVD**.**

5. ***Coagulation markers***

In the past few months, blood clots have emerged as the common factor coalescing many of the mysterious symptoms attributed to COVID-19, a disease that had initially been thought to largely affect the lungs in the form of pneumonia. In the above context, D dimers has emerged as potential marker in predicting COVID-19 severity. D-dimer are degradation products formed from the lysis of cross-linked fibrin with rising levels indicating the activation of coagulation and fibrinolysis [54]. Intriguingly, blood hypercoagulability as a common manifestation among hospitalized COVID-19 patients was evidenced by Terpos *et al*., 2020 with a strong correlation between coagulation abnormalities in PT (prothrombin time), PTT (partial thromboplastin time), FDP (fibrin degradation products), and D-dimer, along with severe thrombocytopenia, and life-threatening DIC (disseminated intravascular coagulation)[55].Correlation between COVID-19 severity and D-dimer levels was also documented by Lippi *et al*., 2020 [56]. It was recorded that around 36-43% of COVID-19 patients had increased levels of D-dimer and may be related to severe complications and death. Supportingly, Tang *et al*., 2020 investigated 207 non-survivor COVID-19 patients and revealed that non-survivors had remarkably higher D-dimer and FDP levels and longer PT at admission compared with survivor [54]. In another retrospective cohort study by Zhang *et al*., 2020 investigating 191 patients found that D dimer levels > 1.0 μg/mL(p=0.0033) were associated with increased mortality among COVID-19 patients. Furthermore, optimum cut-off to predict in-hospital mortality for COVID-19 of 2.0 μg/mL or more on admission was also documented [57]. Additionally, Huang *et al*. found that levels of D-dimer on admission could be used to triage patients into critical care [51].

Based on the evidences from the collected data, the present review provides a substantiate amount of evidences on the role of biomarkers in detection of COVID 19 severity and mortality, thus providing information for diagnosis of high risk patients, triage and early interventions. Yet, further research into the prognostic value of biomarkers is necessary to improve reliability and reproducibility.

Conclusion

In conclusion, data from the presented in this review provides clear insight into the prognostic value of these biomarkers in detection of the COVID-19 infection severity. The present study has some limitations. Data referred in this review includes single centered studies (Wuhan, China). Studies investigating the prognostic markers worldwide are thus needed to validate the findings.

Table 1: Summary of the studies reporting data on biomarkers in COVID-19 detection

References	Study design	Biomarkers investigated and important findings in COVID-19 cases
Qin *et al*., 2020 [19]	Retrospective cohort	Lymphopenia with reduced eosinophils, basophils and higher NLR
Lippi *et al*., 2020 [22]	Meta-analysis	Decreased platelet count
Henry *et al*., 2020 [18]	Meta-analysis	Lymphopenia, increased WBC, lower platelet count
Alsofayan *et al*., 2020 [20]	retrospective	leukopenia, leukocytosis, and lymphopenia, with altered white blood count in severe COVID 19 patients
Cossarizza *et al*., 2020 [24]	Perspective	Decreased T cell count
Chen *et al*., 2020 [29]	Retrospective	Increased levels of AST, ALT, creatinine, LDH, Troponin, D-dimers in non-survivors compared to recovered patients.
Qin *et al*., 2020 [19]	Retrospective	Significantly higher levels of CRP, IL-6, LDH but low LC in severe COVID-19,vs non-severe group

Tan *et al*., 2020 [32]	Retrospective	Strong association found between CRP levels and progression of disease.
Zhou *et al*., 2020; Chen *et al*., 2020 [39, 33]	Retrospective, cohort	Elevated IL-6 with prognostic value at earlier stages identified.
Gong *et al*., 2020 [34]	Perspective, cross sectional	Elevated levels of IL-2R,IL-6, IL-10 in severe COVID 19 cases
Yang *et al*., 2020 [37]	Metaanalysis	Elevated IL-18 and IP-10 and MCP-3 levels found associated with COVID severity
Huang *et al*., 2020 [51]	Retrospective	Increased cTroponin
Shi *et al*., 2020 [52]	Retrospective cohort	Significantly higher levels of hs-TnI in patients who require mechanical ventilation
Zhou *et al*., 2020 [39]	Retrospective	Significantly higher levels of hs-TnI in non-survivors compared to survivors
Lippi *et al*., 2020 [56]	Metaanalysis	Increased coagulability with elevated D-dimers in non survivors
Tang *et al*., 2020 [54]	Retrospective	Markedly elevated levels of D-dimers in deaths with COVID-19 due to abnormal coagulation
Zhang *et al*., 2020 [57]	Retrospective	Optimum cut-off of 2.0 μg/mL of D-dimers to predict in-hospital mortality for COVID-19 was reported

References

1. WHO. Coronavirus (COVID-19) data. The latest data on the COVID-19 global outbreak. Last updated 31st August, 2020. https://www.who.int/data#reports
2. WHO situation report 125 Kingdom of Saudi Arabia-Data as reported till 31st August 2020 http://www.emro.who.int/images/stories/saudi_arabia/saudi-arabia-covid-sit-rep-125-2020-09-01.pdf
3. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, *et al*. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. N Engl J Med. 2020; 382(13):1199-1207.
4. Li Taisheng. Peking Union Medical College Hospital's Proposal for Diagnosis and Treatment of "Novel Coronavirus Infected Pneumonia" (V2.0), Union Medical Journal, 2020, 1:27.
5. Qiuhua W. Disinfection measures for pneumonia epidemic sources of novel coronavirus infection in 2019, Chinese Journal of Disinfection. 2020; 37(1):59-62.
6. Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. J Pharm. Anal. 2020.
7. Zaim S, Chong JH, Sankaranarayanan V, Harky A. COVID-19 and Multi-Organ Response Curr. Probl. Cardiol, 2020, 100618,
8. Su S, Wong G, Shi W, *et al*. Epidemiology, genetic recombination, and Pathogenesis of coronaviruses. Trends Microbiol. 2016; 24(6):490.
9. Stadler K, Masignani V, Eickmann M, *et al*. SARS--beginning to understand a new virus. Nat Rev Microbiol, 2003; 1:209-1.
10. Vashist SK, *et al*. In Vitro Diagnostic Assays for COVID-19: Recent Advances and Emerging Trends. Diagnostics, 2020, 10:202. doi:10.3390/diagnostics10040202
11. Xiong Y, Liu Y, Cao L, *et al*. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. Emerg Microbes Infect. 2020; 9(1):761-770.
12. Klok FA, Kruip M, Van der Meer NJM, *et al*. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res, 2020.
13. Cui S, Chen S, Li X, Liu S, Wang F. Prevalence of venous thromboembolism in patients with severe novel coronavirus pneumonia. J Thromb Haemost, 2020
14. Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, *et al*. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). Clin Infect Dis, 2020.
15. Long *et al*., Antibody responses to SARS-CoV-2 in patients with COVID 19. Nature medicine, 2020.
16. Yang X, Yu Y, Xu J, *et al*. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med. 2020; 8(5):475-481.
17. Chen N, Zhou M, Dong X, *et al*. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020, 395(10223):507-513
18. Henry BM, de Oliveira MHS, Benoit S, *et al*. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. Clin Chem Lab Med, 2020.
19. Qin C, Zhou L, Hu Z, *et al*. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin Infect Dis. 2020. DOI:10.1093/cid/ciaa248
20. Alsofayan *et al*. Clinical characteristics of COVID-19 in Saudi Arabia: A national retrospective study. Journal of infection and Public health. 2020; 13(7):920-25.
21. Arentz M, Yim E, Klaff L, Lokhandwala S, Riedo F, Chong M, *et al***.** Characteristics and outcomes of 21 critically Ill patients with COVID-19 in Washington State**.**J Am Med Assoc, 2020.
22. Lippi G, Plebani M, Henry BM. Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: A meta-analysis. Clinica Chimica Acta, 2020; 506:145-8.
23. Luo S, Zhang X, Xu H. Don't overlook digestive symptoms in patients with 2019 novel coronavirus disease (COVID-19). Clin Gastroenterol Hepatol. 2020
24. Cossarizza A, De Biasi S, Guaraldi G, *et al*. SARS-CoV2, the virus that causes COVID-19. Cytometry. 2020; 97(4):340-343.
25. Ruan Q, Yang K, Wang W, *et al*. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med. 2020; 46(5):846-848
26. Yang M, Ng MHL, Li CK. Thrombocytopenia in patients with severe acute respiratory syndrome (review). Hematology. 2005; 10(2):101-105.
27. Zhou F, Yu T, Du R, *et al*. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet (Lond Engl). 2020; 395(10229):1054-1062
28. Lei F, Liu YM, Zhou F, *et al*. Longitudinal association between markers of liver injury and mortality in COVID-19 in China. Hepatology, 2020.
29. Chen T, Wu D, Chen H, *et al*. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ, 2020, 368:1091.

30. Ferrari D, Motta A, Strollo M, Banfi G, Locatelli M. Routine blood tests as a potential diagnostic tool for COVID-19, Clin. Chem. Lab. Med, 2020.
31. Cheng Y, Luo R, Wang K, Zhang M, Wang Z, Dong L, *et al*., Kidney disease is associated with in-hospital death of patients with COVID-19, Kidney Int. 2020; 97(5):829-838.
32. Tan L, Wang Q, Zhang D, Ding J, Huang Q, Tang YQ, *et al*., Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study, Signal Transduction and Targeted Therapy. 2020; 5(1):1-3.
33. Chen X, Zhao B, Qu Y, Chen Y, Xiong J, Feng Y, *et al*. Detectable serum SARS-CoV-2 viral load (RNAaemia) is closely associated with drastically elevated interleukin 6 (IL-6) level in critically ill COVID-19 patients. Clin. Infect. Dis, 2020. https://doi.org/10.1093/cid/ciaa449
34. Gong J, Dong H, Xia SQ, Huang YZ, Wang D, Zhao Y, *et al*. Correlation Analysis between Disease Severity and Inflammation-related Parameters in Patients with COVID-19 Pneumonia. Med Rxiv, 2020. https://doi.org/10.1101/2020.02.25.20025643
35. Zhou Y, Fu B, Zheng X, Wang D, Zhao C, Qi Y, *et al*. Aberrant pathogenic GM-CSF+ T cells and inflammatory CD14+CD16+ monocytes in severe pulmonary syndrome patients of a new coronavirus, 2020b. bioRxiv. https://doi.org/10.1101/2020.02.12.945576
36. Wen W, Su W, Tang H, Le W, Zhang X, Zheng Y, *et al*. Immune cell profiling of COVID-19 patients in the recovery stage by single-cell sequencing. Cell Discov. https://doi.org/10.1038/s41421-020-0168-9.
37. Yang Y, Shen C, Li J, Yuan J, Wei J, Huang F, *et al*. Plasma IP-10 and MCP-3 levels are highly associated with disease severity and predict the progression of COVID-19. J Allergy Clin. Immunol, 2020. https://doi.org/10.1016/j.jaci.2020.04.027.
38. Herold T, Jurinovic V, Arnreich C, Lipworth BJ, Hellmuth JC, Von Bergwelt-Baildon M, *et al*. Elevated levels interleukin-6 and CRP predict the need for mechanical ventilation in COVID-19. J. Allergy Clin. Immunol, 2020.
39. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, *et al*. Clinical course and risk factors for mortality of adult in patients COVID-19 in Wuhan, China: a retrospective cohort study. Lancet, 2020; 395:1054-1062.
40. Karzai W, Oberhoffer M, Meier-Hellmann A, *et al*. Procalcitonin—a new indicator of the systemic response to severe infections. Infection. 1997; 25(6):329-334.
41. Russwurm S, Wiederhold M, Oberhoffer M, *et al*. Molecular aspects and natural source of procalcitonin. Clin Chem Lab Med. 1999; 37(8):789-797
42. Zhang G, Hu C, Luo L, *et al*. Clinical features and short-term outcomes of 221 patients with COVID-19 in Wuhan, China. J Clin Virol. 2020; 127:104364.
43. Chen T, Dai Z, Mo P, *et al*. Clinical characteristics and outcomes of older patients with coronavirus disease 2019 (COVID-19) in Wuhan, China a single centered, retrospective study. J Gerontol A Biol Sci Med Sci, 2020.
44. Sun D, Li H, Lu XX, *et al*. Clinical features of severe pediatric patients with coronavirus disease 2019 in Wuhan: a single center's observational study. World J Pediatr, 2020.
45. Chen X, Yang Y, Huang M, *et al*. Differences between COVID-19 and suspected then confirmed SARS-CoV10 G. PONTI *ET AL*. 2-negative pneumonia: a retrospective study from a single center. J Med Virol, 2020.
46. Tai W, He L, Zhang X, *et al*. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cell Molecular Immunol, 2020.
47. Hanff TC, Harhay MO, Brown TS, *et al*. Is there an association between COVID-19 mortality and the renin–angiotensin system-a call for epidemiologic investigations? Clin Infect Dis, 2020.
48. Liu Y, Yang Y, Zhang C, *et al*. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Sci China Life Sci. 2020; 63(3):364-374.
49. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, *et al*. Prevalence of comorbidities in the novel Wuhan coronavirus (COVID-19) infection: A systematic review and meta-analysis. Int. J Infect. Dis, 2020; 94:91-95.
50. Mahajan K, Chand Negi P, Ganju N, Asotra S. Cardiac biomarker-based risk stratification algorithm in patients with severe COVID-19. Diabetes Metab Syndr. 2020; 14(5):929-931.
51. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395:497-5.
52. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, *et al*. Association of Cardiac Injury with Mortality in Hospitalized Patients with COVID-19 in Wuhan, China. JAMA Cardiol, 2020
53. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, *et al*. Cardiovascular Implications of Fatal Outcomes of Patients with Coronavirus Disease 2019 (COVID-19). JAMA Cardiol, 2020
54. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost. 2020; 18(4):844-847.
55. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, *et al*. Hematological findings and complications of COVID19. Am J Hematol, 2020.
56. Lippi G, Favaloro EJ. D-dimer is associated with severity of coronavirus disease 2019: a pooled analysis. Thromb Haemost, 2020.
57. Zhang L, Yan X, Fan X, Liu H, Liu X, Liu Z, *et al*., D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19, J Thromb. Haemost, 2020.