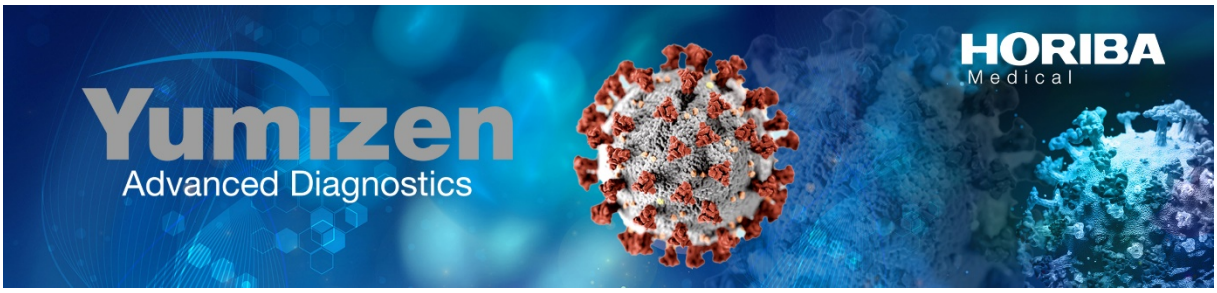

How biological markers could contribute to the monitoring of COVID-19?

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COVID-19 screening, prognosis and severity assessment with biomarkers for management of patients

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Summary

This global epidemic of coronavirus that we are currently experiencing, need an over view on the biological markers that allow the monitoring of COVID-19 disease. After a synopsis of the clinical characteristics and the management of patients, we propose a literature review of the diagnostic tests which include molecular and serological diagnosis. The aim of this document is to show the biological markers involved in screening, triage and prognosis, which involves white blood cells, platelets, D-dimer, CRP and fibrinogen. While acknowledging that these parameters are not exhaustive, they nonetheless represent essential biological markers for the management of this epidemic.

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COVID-19 screening, prognosis and severity evaluation with biomarkers for management of patients

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes the coronavirus disease 2019 (COVID-19), was identified in Wuhan, China at the end of December 2019. Since then, different studies for understanding and reporting the disease have been published, current data sets and conclusions might differ slightly among each other, however, with the ongoing global crisis it is essential to state some observations to provide insightful knowledge to the medical staff for better patient management and decision-making. The scope of this communication document is to review the generalities of the COVID-19, with an emphasis on the assessment of biomarkers, aiming a better understanding on the management of patients with COVID-19. For more comprehensive and detailed information about COVID-19, we strongly recommend the reader to regularly check the updates from the World Health Organization (WHO) (1) and the Centers of Disease Control and Prevention (CDC) (2).

Clinical Characteristics

The COVID-19, an emerging acute respiratory infectious disease, is spread at a low infectious dose mainly by the respiratory tract, by droplets, respiratory secretions and direct contact for a low dose infection (3) (4). Transmission by multiple routes of faecal and blood samples has been reported in patients with severe pneumonia (5).

The incubation period (period between contamination and the appearance of the first symptoms) is 3 to 5 days in general but can extend up to 22 days (6) (7). During this period, the infected subject can be asymptomatic or present mild symptoms, although the risk of transmission is higher in symptomatic patients, transmission of SARS-CoV-2 from asymptomatic persons has been reported (8) (9) (10) (11).

The symptomatic infection spectrum ranges from mild to critical. In a report from the Chinese Center for Disease Control and Prevention presented the following disease severity in more than 44,000 persons with COVID-19 confirmation (12) (2). So, they observe patients with the following criteria: no symptoms, mild to moderate (mild symptoms up to mild pneumonia), severe (dyspnea, hypoxia, or>50% lung involvement on imaging), critical (respiratory failure, shock, or multiorgan system

dysfunction). These symptomatic severe and critical clinical phases are associated with radiological signs, sometimes even in the event of a negative *Polymerase Chain Reaction* (PCR) viral test.

In most laboratory-confirmed studies of this disease, revealed that common clinical manifestations included fever (13) (2) (14) (15), cough, fatigue, sputum production, shortness of breath, sore throat and headache (16). In addition, part of the patients manifested gastrointestinal symptoms, with diarrhea and vomiting (16) (17). Based on information available at the time of writing this paper, despite the uncertainty existing in this documentation and in the observation process, it appears that clinical observations report a significant number of anosmia and ageusia mostly in the non-severe form of the disease (18).

It has been reported that patients without underlying medical conditions had a case fatality of 0.9% (19), however patients with certain pre-existing chronic diseases as well as the elderly are more likely to have lower probabilities of surviving (20). Some reports have shown that children infected with SARS-CoV-2 have milder symptoms compared to adults (2) (14).

Most adults and children infected with SARS-CoV-2 have mild flu-like symptoms and a few patients are in a critical condition and quickly develop an acute respiratory distress syndrome, hemodynamic shock, multiple organ failure with severe thromboembolic disorders leading to death (figure 1) (14).

Management of patients

Considering the epidemics, many Government Healthcare Ministries have recommended that asymptomatic people and persons without severe symptoms can stay at home. Nonetheless, as CDC suggested, 'The decision to monitor a patient in the inpatient or outpatient setting should be made on a case-by-case' (2).

Several categories of patients with COVID-19 must be managed at hospital admission. For example, the Swiss Society of Intensive Care Medicine (March 24, 2020) (21) suggested the following classification of severity among patients with suspicion or confirmation of COVID-19 for a better orientation to Intensive Care Unit (ICU):

- The self-sufficient patients who need no additional oxygen administration required, no organ failure can return to home, they need a medical checkup by the attending physician after 24–48 hours.

- The patients that require a hospital care or oxygen administration, in a COVID 19 ward but without severity criteria, they receive low oxygen administration via nasal cannula/probe, with intermittent monitoring of oxygen saturation and breathing frequencies 3–4 times per day.

- The patients who require an oxygen therapy and continuous monitoring of vital parameters (at least SpO2, ideally blood pressure, heart rate and respiratory rate) in an Intermediate Care Unit.

- The patients with an increasing organ dysfunction (e.g. increasing respiratory failure) must be transferred to the intensive care unit, into a special COVID-19 area.

Moreover, it has been strongly suggested to consider risk factors for the likelihood of developing severe illness. In particular, having pre-existing chronic medical conditions, such as diabetes mellitus, high blood pressure, severe obesity, asthma, and so on. Clinical management of hospitalized patients with COVID-19 should be focused on supportive management of the disease complications: pneumonia, hypoxemic respiratory failure/acute respiratory distress syndrome (ARDS), shock, multiorgan failure, acute thromboembolic disorders. All the complications associated with prolonged hospitalization including secondary nosocomial infection, gastrointestinal bleeding, and critical illness polyneuropathy/myopathy may occur and increase pejorative prognosis (2).

Diagnostic testing

1. Molecular diagnosis

The complete genome of SARS-CoV-2 was sequenced, this virus has a positive-sense RNA genome of 29.9 kb (22) (23) (24). Because it is an RNA virus, molecular diagnosis by quantitative Polymerase Chain Reaction (qPCR) is recommended as an effective pathogen detection method and plays an important role in prevention and control of the current outbreak.

In the case of a public health emergency, proficient diagnostic laboratories can rely on this robust technology to establish new diagnostic tests within their routine services before pre-formulated assays become available. Official protocols available on line provided by the WHO, describes procedures for the detection of SARS-CoV-2 for two RdRp target sequences (IP2 and IP4), developed from the 'Charité institute of virology' in Berlin (25) and validated at 'Institut Pasteur' (Paris).

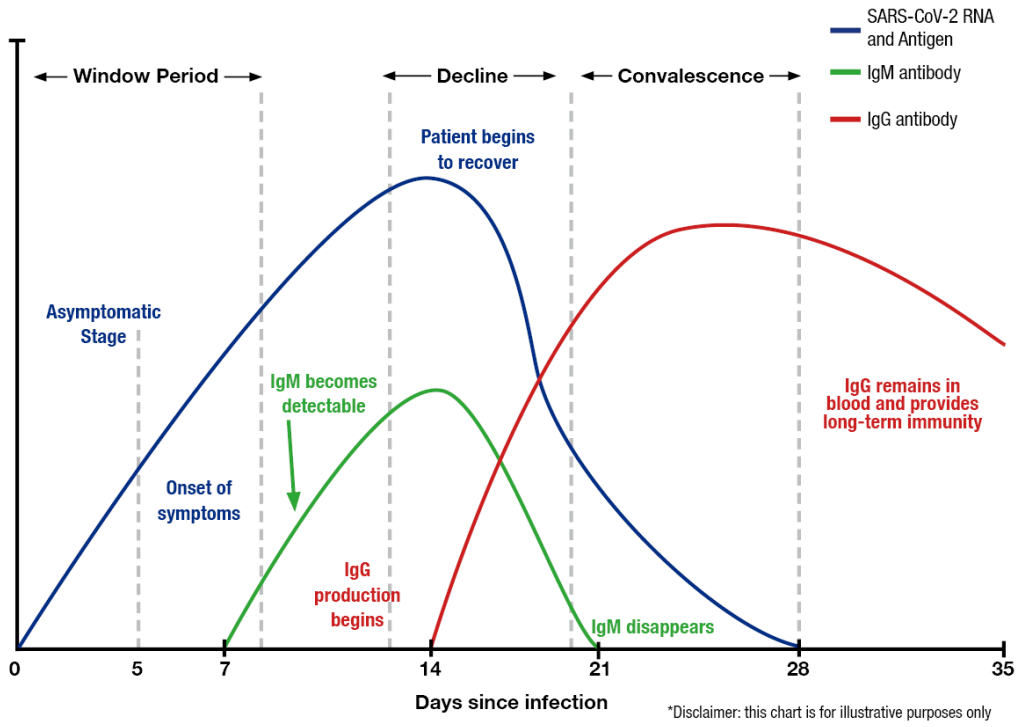
The qPCR is a complex methodology which involves many steps, like sample collection, RNA extraction and retro-transcription/amplification, the latter of which is carried out in one step. This complex workflow may generate false negatives which can be explained by a loss of sensitivity in method detection (26) (27). For this reason, biomarkers can provide insights to patient's management.

A high viral load on presentation of COVID-19 was recorded in patients who were hospitalized shortly after symptom onset. The high viral load on presentation suggests that SARS-CoV-2 can be transmitted easily, even when symptoms are relatively mild. This finding could account for the efficient person-to-person transmission noted in community and health-care settings (28). Quantitative monitoring of viral load in lower respiratory tract samples helps to evaluate disease progression, especially in cases of low viral load (29).

2. Immunology Serology

Last February, internal serological tests were developed to test anti-SARS-CoV-2 in IgM Enzyme-linked immunosorbent assays (ELISA) kits, it was possible to develop them due to amino acid identity with all SARS-CoV-2 markers indicated by Zhou P. et al. (30) (31) Wei Zhang et al. (32) have shown that serological tests can improve detection of carrier cases and therefore should be used in a future epidemiological study (32).

The host humoral response against SARS-CoV-2 including IgA, IgM and IgG response has been evaluated using validated ELISA kits on the recombinant viral nucleocapsid protein (33). The median duration of IgM and IgA antibody detection was 5 days (IQR 3–6), while IgG was detected in 14 days (IQR 10–18) after symptom onset, with a positive rate of 85.4%, 92.7% and 77.9%, respectively. As consequence, humoral response to SARS-CoV-2 can aid to the diagnosis of COVID-19, including subclinical cases (33). The *figure 1* shows serological tests that should be performed on patients and *table 1* shows the clinical significance of combination of qPCR and serological tests.



Source: Diazyme Laboratories, Inc. Retrieved from http://www.diazyme.com/covid-19-antibody-tests

Figure 1: "Variation of the Levels of SARS-CoV-2 RNA and Antigen, IgM and IgG after infection",

Test results			Clinical Significance
RT-qPCR	IgM	IgG	
+	–	–	Patient may be in the window period of infection.
+	+	–	Patient may be in the early stage of infection.
+	+	+	Patients is in the active phase of infection.
+	–	+	Patient may be in the late or recurrent stage of infection.
–	+	–	Patient may be in the early stage of infection. RT-qPCR result may be false-negative.
–	–	+	Patient may have had a past infection, and has recovered.
–	+	+	Patient may be in the recovery stage of an infection, or the RT-qPCR result may be false-negative.

Source: Diazyme Laboratories, Inc. Retrieved from http://www.diazyme.com/covid-19-antibody-tests

Table 1 :"'Clinical Significance of an IgM/IgG Serological Test Result", DIAZYME©

Patients with confirmed COVID-19 pneumonia have typical imaging features, with multifocal lesions in the lung, then Computerized Tomography (CT-scan) can be helpful in early screening of highly suspected cases and in evaluation of the severity and extent of disease (34).

To confirm the diagnosis, the detection by molecular analysis of the viral genome by qPCR appears as the gold standard to detect viruses in respiratory secretions. However, this technology is not available in many healthcare facilities or the external qPCR testing services are saturated, therefore in order to better transfer patients and for taking prompt management decisions, the clinical observations and a comprehensive panel of biological markers might offer a first screening and monitoring scrutiny (35).

Biological Markers for Screening, Triage and Prognosis

Several biomarkers have been observed to be abnormal in COVID-19 infected patients and the relevance of identifying them resides on decreasing the possibility of misdiagnosing severe COVID-19 (36) and to provide more insightful information for better management of COVID-19 patients. Many cohorts of different populations have been reported, principally from China, showing abnormal laboratory assessments consisting mainly of complete blood count, liver and renal function, biochemical and coagulation testing, inflammatory factors, and others.

Hematology Parameters

Different authors have published findings regarding the effect on hematology parameters in COVID-19 infected patients. The vast majority concur on an unbalanced white blood cells panel (37).

White Blood Cells

In the hematological panel, a study that evaluated 1099 positive COVID-19 samples showed on admission and 83.2% of lymphocytopenia (fewer than 1500 cells per mm 3) and leukopenia in 33.7% (16), other authors support these observations especially lymphocytopenia in severe patients (see figure 1) (38) (39) (40) (41) (42) (43) and others reported normal levels of leukocytes (44) (45). Furthermore, neutrophils seem to increase according to the severity of the COVID-19 (14) (17). In a study with 13 patients admitted to ICU and 28 to non-ICU care, the medians of the neutrophil count were 10.6 and 4.4 × 10^9/L, respectively (14). Consequently, due to a significant decrease of lymphocytes and neutrophil increase, the neutrophil-lymphocyte ratio, along with the age of patient, has been suggested as a combined parameter to evaluate the severity of patients with pneumonia caused by COVID-19 for improving risk stratification and management (45) (36).

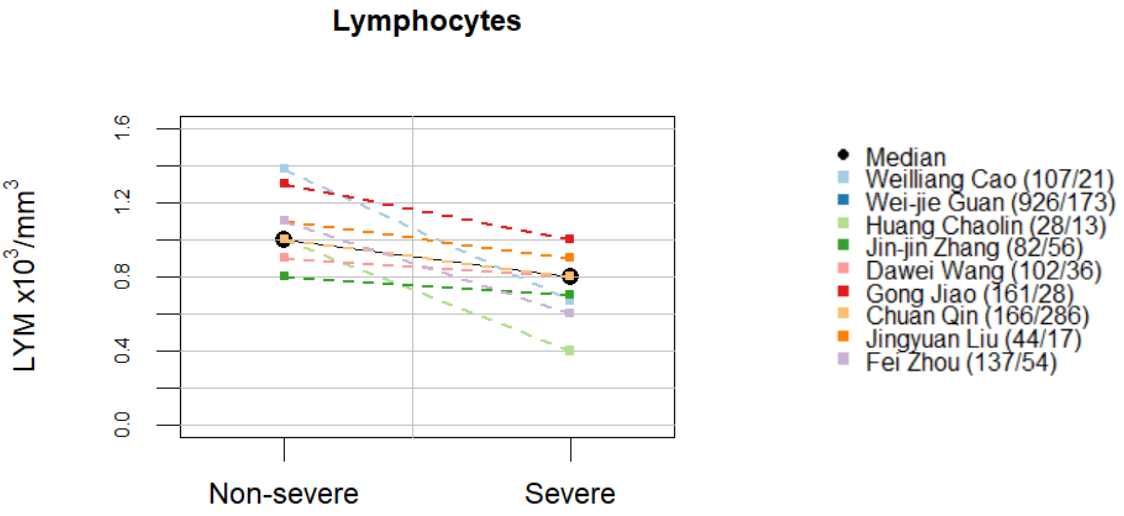


Figure 2: Lymphocyte mean count in non-severe and severe patients from different authors.

The definition of severity varies slightly among references. In all the references inspected, a decrease of lymphocytes was observed in association of COVID-19 severity. The numbers inside the parenthesis are the numbers of non-severe and severe patients per study.

Platelets

A meta-analysis was performed of platelet number in COVID-19 patients with or without severe disease and odds ratio (OR) of thrombocytopenia for severe form of COVID-19 (42). The pooled analysis revealed that platelet count was significantly lower in patients with more severe COVID-19. A subgroup analysis comparing patients by survival, found an even lower platelet count was observed with mortality. In this study, a low platelet count was associated with over fivefold enhanced risk of

severe COVID-19 (42). The low platelet count could be associated with increased risk of severe disease and mortality in patients with COVID-19.

D-dimer

In addition to the unbalanced white blood cell differential, a coagulation testing parameter called D-dimer has been reported to increase in association with the severity of the disease and related to clotting disorders and microthrombotic formation in peripheral blood vessels (46). D-dimer levels in ICU patients (0.6-14.4 mg/L) was higher than non-ICU (0.3-0.8 mg/L) with p-value of 0.0042 (14). In another study, D-dimer shown to be higher in non-survivor patients than survivor patients, particularly a significant difference was observed in the ninth onset day (17), other authors agree that the increasing of D-dimer is linked to the severity of the disease (47) (48) (13) (49).

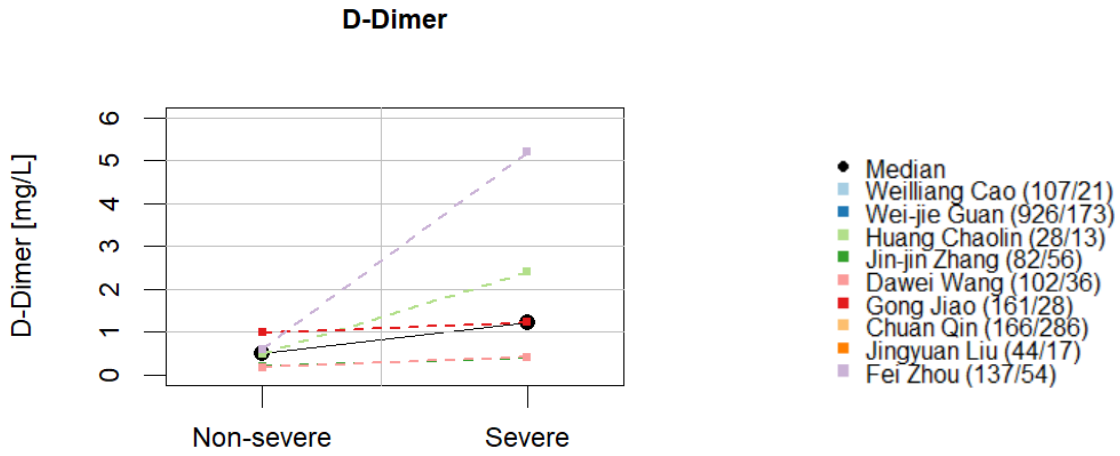


Figure 3: D-dimer mean count in non-severe and severe patients of different authors.

The definition of severity varies slightly among references. In all the references inspected, an increase of D-dimer was observed in association of COVID-19 severity. In (47) & (49) elevation of D-dimer was associated with the survival of infected patients. The numbers inside the parenthesis are the numbers of non-severe and severe patients per study.

CRP

Although C-reactive protein (CRP) does not normally elevate significantly in mild viral respiratory infections, levels have shown to increase in severe cases of avian influenza H1N1 and H7N9, and during the SARS epidemic in 2003. A similar significant increase of CRP has also been reported in COVID-19 patients. COVID-19 can cause mild or highly acute respiratory syndrome with consequent release of pro-inflammatory cytokines, stimulating macrophages and hepatocytes to produce CRP in higher amount.

CRP is the inflammatory marker that has more relevance than other inflammatory markers for assessing the severity of COVID-19 from current literature review (50) (36) (51). For example, in 56.4% of the non-severe cases had a CRP value higher or equal than 10 mg/L, however, in severe

cases, the percentage was 81.5%. In comparison, non-severe and severe cases with PCT ≥ 0.5 ng/ml had a corresponding 3.7% and 13.7% of the samples, respectively (16). In a study comparing 82 non-severe patients and 56 severe patients, the p-values of CRP and pro-calcitonin (PCT) medians were less than 0.001 (52), however, in other articles it has been reported PCT having a normal level (48).

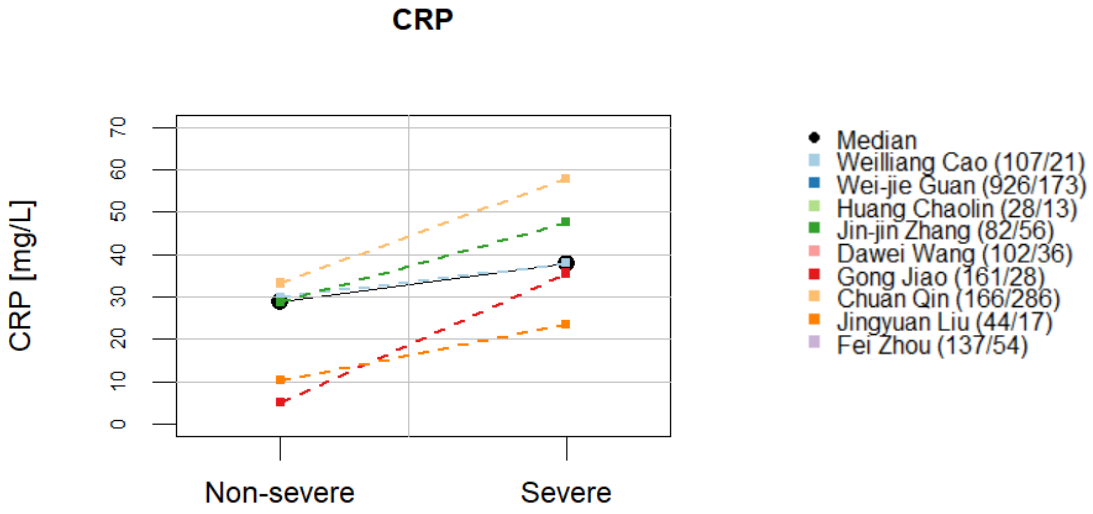


Figure 4: CRP mean count in non-severe and severe patients of different authors.

The definition of severity varies slightly among references. In all the references inspected, an increase of CRP was associated with COVID-19 severity.

Fibrinogen

Fibrinogen degradation products (FDP), and Fibrine (FIB) were found to be higher than in a control healthy population, thus confirming earlier similar findings. Reported data from 94 patients show that FDP (33.83 vs. 1.55 mg/L; $p<0.001$) were higher in patients than those in controls, FIB values in SARS-CoV-2 patients were also higher than those in the control group (5.02 vs. 2.90 g/L; $p<0.001$). The analysis of blood coagulation in COVID-19 patients seems to be clearly deranged compared with a healthy control population. More specifically, FDP, and FIB values were found to be significantly increased (49).

Conclusion

The COVID-19 should be diagnosed using clinical aspects and qPCR as suggested by the WHO. Relevant people and especially healthcare decision makers must be aware on the latest updates by local and global healthcare authorities to follow corresponding guidelines. This review tries to present a summary of different biomarkers providing extra information for a better assessment of COVID-19. During this epidemiological crisis, a strong screening process on the severity of the patient and an informed prognosis of COVID-19 will certainly relieve saturation of specialized facilities and improve the clinical management of patients infected with COVID-19.

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