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CYTOKINES, COAGULATION PROFILE AND HAEMATOLOGICAL CHANGES IN COVID 19 PATIENTS AS INDICATORS OF THEIR HEALTH STAUS: A REVIEW

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ABSTRACT
Covid 19 has drastically changed approaches to many things ranging from health systems, the economy of the world, the social life, working conditions, education systems, safety and challenged continued existence of mankind in the planet earth. During the incubation period, usually ranging from 1 to 14 days, and during the early phase of the disease, when non-specific symptoms are present, peripheral blood leukocyte and lymphocyte counts are normal or slightly reduced. Following viraemia, SARS-CoV-2 primarily affects the tissues expressing high levels of ACE2 including the lungs, heart and gastrointestinal tract. Approximately 7 to 14 days from the onset of the initial symptoms, there is a surge in the clinical manifestations of the disease with a pronounced systemic increase of inflammatory mediators and cytokines, which may even be characterized as a cytokine storm. Lymphopenia is a common finding in patients with COVID-19 infection, and is believed to represent a defective immune response to the virus. The haematological parameters should be monitored carefully to find out if the patients need blood transfusion and the alterations in the coagulation profile and cytokines.

INTRODUCTION
Covid 19 has drastically changed approaches to many things ranging from health systems, the economy of the world, the social life, working conditions, education systems, safety and challenged continued existence of mankind in the planet earth. A lot of things have revolutionized in humans since the Covid 19 pandemic in such a way that the world powers were challenged also. The health institutions were overwhelmed with sick persons with high mortality and morbidity rates in different countries. Nigeria is part of the world the disease is ravaging and making uncomfortable. I t was reported that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) resulting to coronavirus disease 2019 (COVID-19) has quickly changed from an epidemic outbreak in Wuhan, China1 into a pandemic infecting more than 1 million persons across the globe, whereas billions of citizens are affected by ways of social distancing and the socioeconomic burden of the pandemic. SARS-CoV-2 is nearly 80% similar to SARS-CoV and affects host human cells by linking to the angiotensin-converting enzyme 2 (ACE2) receptor (Zhu *et al.*, 2020). Even though it is well reported that COVID-19 is mainly presented as a respiratory tract infection, emerging records show that it should be referred as a systemic disease involving multiple systems namely cardiovascular, respiratory, gastrointestinal, neurological, haematopoietic and immune system (Driggin I *et al.*, 2020; Bangash *et al.*, 2020; Mehta *et al.*, 2020). Death levels of COVID-19 are below SARS and Middle East Respiratory Syndrome (MERS); though, COVID-19 is more deadly than seasonal flu (Wu and McGoogan, 2020). Tang *et al.* (2020) and Madjid *et al.* (2020) opined that aged persons and those with comorbidities are at raised danger of mortality from COVID-19, but younger perosns without major fundamental illneses may alsoshow with potentiallydeadly impediments such as fulminant myocarditis and disseminated intravascular coagulopathy (DIC). Also, interleukin 6 has been reported to predict the death rate in Covid 19 patients. So, it becomes imperative to carry out a study in Nigeria to determine the level of interleukin 6 to predict mortality rate and help to control the spread and loss of life by Covid 19.

Full Blood Count and Biochemistry Findings: Correlation with Prognosis
During the incubation period, usually ranging from 1 to 14 days, and during the early phase of the disease, when non-specific symptoms are present, peripheral blood leukocyte and lymphocyte counts are normal or slightly reduced. Following viraemia, SARS-CoV-2 primarily affects the tissues expressing high levels of ACE2 including the lungs, heart and gastrointestinal tract. Approximately 7 to 14 days from the onset of the initial symptoms, there is a surge in the clinical manifestations

of the disease with a pronounced systemic increase of inflammatory mediators and cytokines, which may even be characterized as a "cytokine storm" (Li *et al.*, 2020). At this point, significant lymphopenia becomes evident. Although more in-depth research on the underlying etiology is necessary, several factors may contribute to COVID-19 associated lymphopenia. It has been shown that lymphocytes express the ACE2 receptor on their surface (Xu *et al.*, 2020); thus SARS-CoV-2 may directly infect those cells and ultimately lead to their lysis. Furthermore, the cytokine storm is characterized by markedly increased levels of interleukins 6 (IL-6) and tumor necrosis factor (TNF)-alpha, which may promote lymphocyte apoptosis (Singh *et al.*, 2014; Liao *et al.*, 2020; Aggarwal *et al*, 1999). Substantial cytokine activation may be also associated with atrophy of lymphoid organs, including the spleen, and further impairs lymphocyte turnover (Chan *et al.*, 2020).

Guan et al. provided data on the clinical characteristics of 1,099 COVID-19 cases with laboratory confirmation during the first two months of the epidemic in China (Guan *et al.*, 2020). On admission, the vast majority of patients presented with lymphocytopenia (83.2%), whereas 36.2% had thrombocytopenia, and 33.7% showed leukopaenia. Specifically, Huang *et al*. (2020) and Wang *et al*. (Wang *et al.*, 2020) highlighted an association between lymphopaenia and need of ICU care, whereas Wu et al. (2020) showed an association between lymphopaenia and acute respiratory distress syndrome (ARDS) development. Specifically, Wu *et al* (2020) retrospectively analyzed possible risk factors for developing ARDS and death among 201 patients with COVID-19 pneumonia in Wuhan, China (Wu *et al.*, 2020). Increased risk of ARDS during the disease course was significantly associated with increased neutrophils ($p<0.001$), decreased lymphocytes ($p<0.001$) in a bivariate Cox regression analysis. Increased neutrophils ($p=0.03$) were associated with increased risk of death (Wu *et al.*, 2020).

Epidemiology and clinical features of the 2019-2020 COVID-19 pandemic

The current COVID-19 pandemic originated in December 2019 in the city of Wuhan, the capital of the Hubei province of China. Despite efforts to contain its spread, the epidemic spread to numerous other countries in Asia and by January 2020 infected patients were identified in Europe (Lu *et al.*, 2020). On March 11 the World Health Organization (WHO) declared a pandemic: at this point there were an estimated 118,000 cases in 114 countries, resulting in 4,291 reported deaths. According the WHO, as of April 9, there were an estimated 1,436,198 cases in 212 countries and territories, resulting in 85,522 reported deaths. The countries with the largest numbers of confirmed cases were the United States (395,030 cases), Spain (146,690 cases), Italy (139,422 cases), Germany (108,202 cases) and China (83,249 cases) (WHO, 2020). Early in the pandemic Zhu et al isolated and characterized the virus (preliminarily called 2019-nCoV, renamed SARS-CoV2, and finally COVID-19). Like the viral agents responsible for the severe acute respiratory syndrome (SARS) outbreak of 2002-2003 and the Middle East respiratory syndrome (MERS) outbreak of 2012-2013, COVID-19 is a coronavirus. Coronaviruses have a positive-sense single-stranded RNA genome and a helical capsid with an envelope composed of a lipid bilayer (Zhu *et al.*, 2020; Perlman, 2019). Sequence analysis of the genome of COVID-19 revealed that it has a strong homology to SARS-like coronaviruses that normally infect bats, and for this reason the pandemic is believed to be of zoonotic origin (Lippi and Plebani, 2020). Like the SARS and MERS outbreaks, the predominant clinical features demonstrated by individuals infected during the COVID-19 pandemic are respiratory. Following an incubation period of up to 2 weeks duration, patients become symptomatic. Fever (identified in ~99% of patients), cough (~50% of patients), and respiratory difficulty (~33% of patients) are the most common complaints. Approximately 80% of infected individuals have mild to moderate symptoms. The remainder has severe enough disease to necessitate hospitalization. Among severely ill individuals, the most severe complications are acute respiratory distress syndrome/ diffuse alveolar damage. Several co-morbidities have been proposed which predispose patients to severe disease. Zhou et al addressed a wide range of co-morbidities and laboratory abnormalities potentially impacting prognosis in COVID-19 patients. In their multivariate analysis the following features were associated with increased odds of death: older age; higher sequential organ failure assessment (SOFA) score (a scoring system based on PaO2/FiO2, Glasgow coma scale, mean arterial pressure, serum bilirubin, platelet count, and creatinine); and d-dimer greater than 1 μg/mL at admission (Zhou *et al.*, 2020).

The disease trajectory and percentage of severe cases stands in contrast to patients identified in the SARS and MERS outbreaks, which had a shorter incubation and a higher fraction of severe cases and deaths from disease. As a result of the longer incubation period and presumed lower fatality rate, COVID-19 has infected a significantly larger number of individuals than those affected by the SARS and MERS outbreaks (Lippi and Plebani, 2020).

A recently identified clinical phenomenon is reactivation of COVID-19 infection in a subset of patients following recovery from initial disease. Although it has not yet been widely reported in the peer-review medical literature, a report by Ye et al identified reactivation in 5 patients from a cohort of 55 patients from China (Ye *et al.*, 2020). Notably, influenza and H7 avian influenza virus were excluded by additional testing, but repeat testing for COVID-19 does not appear to have been performed. As of publication, all patients are alive without evidence of pneumonia. The haematologic

characteristics of this group of patients with COVID-19 have not yet been definitively explored.

Haematologic parameters of patients with COVID-19 infection

On the basis of studies conducted in China and elsewhere, the clinical haematology laboratory plays an important role by providing the clinical team a number of useful prognostic markers. Although information is in some cases based on the results of limited amount of data and should be validated with additional studies, the available findings clearly establish the clinical hematology laboratory as an important partner in the triage and management of affected patients. Apart from RT-PCR testing for the organism, laboratory tests have not been assessed with regard to their sensitivity or specificity for the diagnosis of COVID-19, although their values as prognostic indicators have been established. A summary of the major hematologic features of importance in COVID-19 infected patients follows.

Lymphopenia

Lymphopenia is a common finding in patients with COVID-19 infection, and is believed to represent a defective immune response to the virus (Lippi and Plebani, 2020). In their early study of 41 adults with RT-PCR confirmed COVID-19 infection, Huang et al noted that lymphopenia (defined as an absolute lymphocyte count $<1.0 \times 10^9$/L) was seen in 26 (63%) of patients (Huang *et al.,* 2020). This is typical for the series reported in the medical literature. A recent meta-analysis noted that 35–75% of patients developed lymphopenia, which was a more frequent feature of patients who died of disease (Lippi and Plebani, 2020). In their analysis of 67 COVID-19 patients from Singapore, Fan *et al* identified an lymphocyte count of $<0.6 \times 10^9$/L being predictive for admission to the intensive care unit (ICU) (Fan *et al.,* 2020).

There appears to be some geographic variability in the percentage of COVID-19 patients who present with lymphopenia. For example, a paper reporting a series of COVID-19 patients from Singapore identified a much lower percentage of patients with lymphopenia, as did a retrospective analysis of COVID-19 patients from Zhejiang Province, which is located ~450 miles from Wuhan (Fan *et al.,* 2020; Xu *et al.,* 2020).Conversely, in a series of patients from Italy, patients presenting in the emergency department demonstrate lymphopenia in many cases (Buoro *et al.,* 2020). The reasons for these and similar discrepancies are unclear, although they are likely multifactorial. Because of the apparent viral genomic mutations, it is possible that the immunologic response to the virus may change as the pandemic expands into other countries. Another possibility is that testing of patients is nonuniform and, depending on the time of presentation, the extent of lymphopenia may vary. A careful review of reported data for these issues is therefore recommended.

In children, lymphopenia is much less common. In their meta-analysis of 66 cases reported in the Chinese literature, Henry et al identified lymphopenia in 3% of patients. This is in contrast to other similar viral infections, such as SARS, in which lymphopenia was a much more common finding in children (Henry *et al.,* 2020).

Leukocytosis

Leukocytosis, irrespective of whether it represents a neutrophilia, lymphocytosis, or both, is noted in a minority of COVID-19 infected patients, and appears to herald bacterial infection or superinfection (Lippi and Plebani, 2020). A meta-analysis of the extant literature noted that leukocytosis was identified in 11.4% of patients with severe disease compared to 4.8% of patients with mild to moderate disease (odds ratio [OR], 2.54; 95% confidence interval [CI], 1.43–4.52) (Lippi and Plebani, 2020).

Neutrophilia

The data on neutrophilia are incomplete and have not been widely addressed in the literature. The available data suggest that neutrophilia is an expression of the cytokine storm and hyperinflammatory state which have an important pathogenetic role in COVID-19 and related infections such as SARS (Chen *et al.,* 2020; Mehta *et al.,* 2020; Qin *et al.,* 2020). Cytoplasmic and nuclear morphological anomalies, from hyposegmented nuclei to apoptosis, have been described in circulating granulocytes at the time of hospital admission, possibly in relation with the hyperinflammatory state with cytokine storm. They usually precede the increase of reactive lymphocytes (Zini *et al., 2020*). Neutrophilia may also indicate superimposed bacterial infection (Lippi and Plebani, (2020). For example, Fan et al noted that neutrophilia is common in patients treated in the ICU during hospitalization (11.6 vs 3.5x109/L) (Fan *et al.,* 2020).

Markers of systemic inflammation

Another potential application of data derived from the CBC would be to use formulas such as neutrophil-tolymphocyte ratio (NLR), platelet-to-lymphocyte ratio, and monocyte-to-lymphocyte ratio to act as surrogates to assess the extent of systemic inflammation. Although extensive study is at this point lacking, Qin *et al* have reported an increase in NLR in patients with severe disease compared to those without (Qin *et al.,* 2020).

Thrombocytopenia

Thrombocytopenia is an important indicator of severe disease in COVID-19 patients, as highlighted by a recent review of the available peer reviewed data. This is not surprising, since platelet count is used by scoring systems such as the Multiple Organ Dysfunction Score (MODS), Simplified Acute Physiology Score (SAPS) II, and Acute Physiology and Chronic Health Evaluation (APACHE) II, and thrombocytopenia is an indicator of severe disease in these systems (Lippi *et al.,* 2020). A

meta-analysis pooling data from 9 studiesshowed that thrombocytopenia has been reported in a majority of patients. This is similar to data reported in the SARS outbreak, in which thrombocytopenia was reported in ~55% of cases and correlated with increased risk of severe disease (Yang *et al.,* 2005; He *et al.,* 2003). In patients with severe infection, thrombocytopenia is identified in up to 57.7% of patients, vs. 31.6% of patients with less significant COVID-19 symptoms (OR 2.96, 95% CI, 2.07–4.22) (Lippi and Plebani, (2020).

The use of platelet count in conjunction with other factors associated with severe disease has to our knowledge not been reported for COVID-19 patients, although it has been revealed to be of use in SARS. For example, Zou *et al* reported that platelet count, in conjunction with hypoxemia, were used in prognostic model for SARS that predicted severe disease with 96.2% accuracy (Zou *et al.,* 2004). In addition, elements of the expanded CBC useful in evaluation of sepsis, such as mean platelet volume and reticulated platelet count, have not to our knowledge been reported in the COVID-19 literature, but may be of use in risk stratification and clinical decision making.

Coagulation parameters

Only rare articles are published related to coagulation parameters in COVID-19 patients, mainly from China (Tang *et al.,* 2020; Yin *et al.,* 2020). A subset of severe pneumonia patients develop viral sepsis, disseminated intravascular coagulation (DIC) and multiorgan failure.(26) Coagulation parameters show abnormal results related to sepsis or DIC. Prothrombin time (PT), an assay used to evaluate the extrinsic and common coagulation pathways, and D-dimer are useful indicators of prognosis and severity of disease in COVID-19 (Perlman, 2019). In a study with 183 coronavirus pneumonia, PT, activated partial thromboplastin time (APTT), fibrinogen, antithrombin, FDP and D-dimer were consecutively measured during 2-week hospitalization. The overall mortality was 11.5%. The non-survivors demonstrated significantly higher D-dimer and fibrin degradation product (FDP) levels, and longer PT and APTT compared to survivors on admission. The fibrinogen and antithrombin levels were significantly reduced in non-survivors during hospitalization, and D-dimer and FDP are markedly elevated in all non-survivors by the late hospitalization, which suggested a common coagulation activation, disregulated thrombin generation, impaired natural anticoagulants and fibrinolysis. Overt DIC (5 or higher points according to the International Society on Thrombosis and Haemostasis diagnostic criteria for DIC) (Taylor *et al.,* 2001) was developed more frequently in non-survivors than survivors (71.4% vs 0.6%, respectively) in median 4 days from admission (Lillicrap, 2020). In addition, several critically-ill patients have been reported to develop coagulopathy, antiphospholipid antibodies and increased arterial and venous thrombotic events such as cerebral infarction (Zhang *et al.,* 2020). Early recognition of these abnormal coagulation results will be useful to predict the disease severity, support to guide the therapy, and improve the patients' clinical outcome (Tang *et al.,* 2020).

Laboratory confirmation of COVID-19 infection

Because of the rapid spread of the COVID-19 pandemic, affected countries have taken a heterogeneous and evolving approach to diagnosis of infection in patients and continue to have different and in some cases evolving strategies to determine what segments of the population should be tested. The molecular diagnosis of COVID-19 infection has been the subject of numerous scientific publications, many of which are beyond the scope of this review. Briefly, 2 major diagnostic approaches have been implemented in a majority of countries, both using RT-PCR. The first, which has the approval of the WHO, is that of Corman *et al*, which has 3 viral genes (*E*, *RdRp*, and *N*) as targets (Corman *et al.,* 2020; Lippi *et al.,* 2020). Screening is conducted using an assay directed at the *E* gene and is confirmed by testing for the *RdRP* and *N* genes. A second assay was developed by the Centers for Disease Control and Prevention (CDC) in the United States and uses a combined assay for the viral *N1/2/3 gene* with the *RNase P* gene as a control assay. This latter approach is the basis for many of the inlaboratory testing approaches developed by medical centers and commercial laboratories in the United States (Lippi *et al.,* 2019). Because of the rapid implementation of diagnostic testing for COVID-19, some features have become obvious only after widespread testing of patient populations. The first of these is the apparent suboptimal number of false negative RT-PCR results. In recent studies a small number (~3%) of patients with computerized tomography (CT) findings strongly suggestive of COVID-19 infection initially were negative using the RT-PCR based testing. In at least one study all of the initially negative patients had a positive result on repeat testing after a mean interval of ~5.0 days (Xie *et al.,* 2020). This feature is understandable in view of the known disease trajectory in patients with severe COVID-19 disease. Since the mean incubation period is approximately 6 days, and viral load significantly increases during this period, testing conducted early in the symptomatic period may be falsely negative. Similarly, RT-PCR results may be falsely negative in recovering infection when patients are still presumably infectious, again due to the same features of disease kinetics. Both these scenarios have obvious negative implications for the use of molecular-based testing alone as the sole means of controlling the spread of infection (Lippi *et al.,* 2019).

At least 2 important factors make the study of the epidemiologic features of COVID-19 challenging. The first is the lack of a uniformly applied diagnostic approach. Second, different nations have taken radically different approaches to population screening. Extreme examples of this heterogeneity are South Korea, a

relatively small nation, which has tested over 65,000 individuals compared to the United States which was delayed in implementing RT-PCR which at the time of preparation of this manuscript has tested a much smaller number of individuals (Lippi and Plebani, 2020).

As the pandemic matures, it will likely be useful to identify the overall number of individuals who have been exposed to COVID-19 and have developed a successful immune response. Since approximately 30% of adults and possibly a larger percentage of children have clinically silent infection, a mass screening approach of the general population may be informative. A combined IgG/IgM immunoassay has been developed which can achieve this goal in a simple and cost-effective manner (Lippi *et al.*, 2019).

CONCLUSION

Covid 19 has drastically changed approaches to many things ranging from health systems, the economy of the world, the social life, working conditions, education systems, safety and challenged continued existence of mankind in the planet earth. During the incubation period, usually ranging from 1 to 14 days, and during the early phase of the disease, when non-specific symptoms are present, peripheral blood leukocyte and lymphocyte counts are normal or slightly reduced. Lymphopenia is a common finding in patients with COVID-19 infection, and is believed to represent a defective immune response to the virus. The haematological parameters should be monitored carefully to find out if the patients need blood transfusion and the alterations in the coagulation profile and cytokines.

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