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SARS-CoV-2 RT-PCR Ct Value and Laboratory Tests: Clinicopathologic Characteristics among Adult Filipino Inpatients diagnosed with COVID-19 in a Tertiary Medical Center

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ABSTRACT

Introduction. The role of the laboratory during the COVID-19 pandemic is not limited to just diagnosis of the disease, but also in clinical decision-making, by providing information on relevant laboratory biomarkers. Clinicians also use Ct value to guide patient management. There are limited studies available locally regarding the significance of Ct value and pertinent laboratory biomarkers in COVID-19 patients. This study aimed to assess the aforementioned laboratory data, along with the clinicopathologic characteristics of affected patients, and determined if this information may be useful for robust clinical decision-making.

Methodology. In this retrospective analytic study, we identified 325 out of 1,049 adult Filipino inpatients diagnosed with COVID-19 and analyzed their Ct values and pertinent laboratory biomarkers such as neutrophil and lymphocyte count, platelet count, LDH, ferritin, procalcitonin, CRP, AST/SGOT, ALT/SGPT, PT/INR, and D-dimer, and correlated them with the severity of the disease.

Results. Two hundred twenty (67.7%) patients had non-severe disease, while 105 (32.3%) had severe disease. Lower Ct values of ORF1ab (median = 26.4) and N (median = 24.8) genes were seen in the severe group compared to the non-severe group and were found to be significant ($p<0.001$). Laboratory markers (neutrophil, platelet counts, LDH, ferritin, procalcitonin, CRP, AST, PT/INR, and D-dimer) were associated with severe COVID-19. On the other hand, ALT was not associated with severe disease.

Conclusion. The laboratory biomarkers together with Ct value and overall clinical picture may provide valuable information to physicians for more robust clinical decision-making.

Key words: COVID-19, cycle threshold, laboratory biomarkers, SARS-CoV-2, RT-PCR

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INTRODUCTION

COVID-19 is a disease caused by a novel beta coronavirus, SARS-CoV-2, which was first discovered in Wuhan, China last December 2019 and has subsequently spread all over the globe.[1] Based on the World Health Organization (WHO) situation report as of March 8, 2023, the Philippines has had 4,077,302 confirmed cases, with 98.14% recovered cases and 1.86% fatalities.[2] Among the severe (13%) and critical (5.5%) cases, the most affected were individuals aged 60-69 years (26%) followed by those aged 70-79 years (22%), and around 14% of deaths involved those aged 80 years and up.[2]

Currently, both globally and in the Philippines, the most widely used method to confirm the diagnosis of COVID-19 is by detecting viral nucleic acids in respiratory tract samples with real time reverse transcriptase polymerase chain reaction (RT-PCR).[3] The WHO recommends that SARS-CoV-2 RT-PCR tests detect certain viral genes, such as *ORF1ab* (Open Reading Frame) and *N* (nucleocapsid), which were discovered early in the viral genome, alongside *S* (spike) and *E* (envelope) genes.[4] These genes in particular code for major structural proteins in the SARS-CoV-2 virus, which help to differentiate it from other members of the coronavirus family.[5] The data obtained by RT-PCR test is reported as cycle threshold (Ct) value, which

represents the number of times an amplification of a target gene occurs prior to detection.[6,7] Ct values in theory can represent an indirect measurement of viral load, and their relationship is inversely proportional, such that a higher viral load would be represented by a lower Ct value.[6–8] The RT-PCR for the detection of SARS-CoV-2 viral RNA is a qualitative test, i.e., a Ct value less than the determined cut-off value is considered positive and the absence of a Ct value or more than the set cut-off is considered as negative.[6] Although RT-PCR for COVID-19 testing is a qualitative test, the majority of the clinicians use the Ct value in the management of patients.[9,10] Current data suggest that a lower Ct value may be associated with poor prognosis, abnormal biomarkers, and generally a worse clinical outcome, though this is not consistent, as Ct values can be affected by other variables inherent to the test itself or by individual patient variables.[7,8,11]

Of note is the multitude of variables that affect the Ct value. The majority of these variables are pre-analytic, which can include patient factors, disease factors, sampling methods, specimen transport and age.[12] With regards to sampling technique, the laboratory cannot distinguish whether it is of an anterior nasal swab, a mid-turbinate swab, or a combined oro/nasopharyngeal swab. The lack of a standardized sampling method may contribute to the variability of the Ct value because of the differences in sample quality, with lower sensitivities found in nasal swabs, throat swabs, and saliva. RNA material concentration varies in specimen type and therefore has an influence on the Ct value.[12,13]

The clinical picture of COVID-19 varies widely, and can range from asymptomatic persons, or those with a mild upper respiratory tract illness, up to severe or critical cases of acute respiratory distress requiring mechanical ventilation with high morbidity and mortality (up to 94%).[3,14]

Correlation with the patient's history is important when dealing with Ct values. It was reported that the highest viral load belonged to the presymptomatic stage. Thus, the timing of specimen collection must be noted by the clinician when interpreting the Ct value.[15] Another study has shown that Ct values are highest among asymptomatic infections, with the values decreasing as the patients become presymptomatic and symptomatic. This supports the idea that Ct values reflect viral load and disease severity.[16]

In addition, some patients may exhibit prolonged viral shedding, with low Ct values persisting particularly in patients with more severe disease, the elderly and immunocompromised population, and in those without proper antiviral treatment. Those with more severe disease had detectable Ct values up to 28 days after the onset of symptoms.[17] It is postulated that a less robust immune response in the elderly and immunocompromised populations may contribute to the persistence of the virus, and hence the persistence of detectable viral particles upon testing.[17,18]

There are also some cases wherein patients who meet hospital discharge criteria and are sent home, still have positive RT-PCR results. Their Ct values gradually increase until they test negative. However, for some patients, symptoms recur, and Ct values also remain low, suggesting re-infection. Hence, post-discharge patient monitoring is important in curbing the spread of COVID-19.[19]

In the diagnosis of COVID-19, clinical assessment of patients are important, and this is supplemented by the provision of biomarkers or laboratory markers which provide clinicians with objective information that can impact patient care.[20] Different biomarkers are recommended for use, including hematologic, immunologic, inflammatory, coagulation, and biochemical markers, which reflect the underlying pathophysiology of SARS-CoV-2 infection.[14,20] With the emergence of studies involving biomarkers in COVID-19 disease, it is being increasingly recognized that this is not confined to the respiratory system, but instead is a disease that involves multiple organ systems.[14,20,21] The role of the laboratory during this time is not only limited to the detection of this novel disease entity, but also to aid patient management by reporting of pertinent laboratory parameters or biomarkers. These biological substances can be objectively measured and evaluated, and give clinicians insight into disease progression, patient prognosis, and therapeutic monitoring of medical and pharmacological interventions.[14,22,23]

To our knowledge, there are limited studies available locally regarding the significance of Ct value and pertinent laboratory biomarkers in COVID-19 patients.[7] In this study, we assessed these laboratory data along with the clinicopathologic findings of affected patients and determined if this information may be useful for clinicians for more robust clinical decision-making.

METHODOLOGY

Population and sampling

In this retrospective study, we included adult (19 years old and above) Filipino inpatients diagnosed with COVID-19 by RT-PCR in The Medical City, a tertiary hospital in Pasig City with a 500-bed capacity, from April 2020 to December 2020. Pediatric patients (aged 18 years and below), asymptomatic COVID-19 patients, patients without available laboratory work-up, and those who consulted the institution on an outpatient basis were excluded from the study (total excluded, n = 724).

The patient demographic data, clinical diagnosis and other pertinent medical history were obtained by review of electronic medical records. The initial SARS-CoV-2 RT-PCR result (including the Ct values of *ORF1ab* and *N* genes) and other laboratory workup were retrieved using the laboratory information system (LIS).

RNA extraction for all samples was performed using the *GeneFinder EX-MATE 32* instrument. The corresponding extraction kit is the *GeneFinder* Viral DNA/RNA Extraction kit. Samples were treated with a lysis buffer in order to free the nucleic acids, which then get bound to the magnetic particles. Washing was done to separate the unneeded lysed cellular components, leaving only the desired eluate containing RNA. The eluate was then processed for RT-PCR.

The test kit used for the RT-PCR assay was Sansure Biotech, which targets viral ORF1ab and N genes. Fluorescent dyes were used in order to distinguish the amplifications of the different targets. The dyes used were FAM for *ORF1ab* gene, ROX for *N* gene, and Cy5 for *RNase P* gene (internal control). The assay has a manufacturer-declared positive agreement rate (sensitivity) of 94.34% (95% CI: 84.34 ~ 98.82%) and a negative agreement rate (specificity) of 98.96% (95% CI: 96.31 ~ 99.87%). All samples were run in the *Bio-rad CFX thermal cycler* instrument. The entire process was performed in accordance with the manufacturer's recommendations. Batch runs of RT-PCR were considered valid if the negative controls had no amplifications for all three targets, and if the positive controls had amplifications in all targets with Ct values of 40 or lower. Individual samples were considered valid (have adequate extracted RNA) if they have amplification of their internal control (Cy5 channel) with Ct value of 40 or lower. A positive result was rendered if the sample had any amplification with Ct value or 40 or lower in either FAM or ROX channel. Negative results were rendered for samples that had no amplification in both FAM and ROX channels.[24]

A cut-off of 30 was used to classify Ct value, wherein Ct values below 30 were considered low, and Ct values above 30 were considered high.[25,26] Other laboratory parameters included in the study were based on the Interim Guidance on the Clinical Management of COVID-19 Version 3.1 document, which recommended the following parameters to support the diagnosis of COVID-19: neutrophil count, lymphocyte count, platelet count, lactate dehydrogenase (LDH), ferritin, procalcitonin, c-reactive protein (CRP), aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), PT/INR, and D-dimer.[3]

The identified patients included in the study were further subdivided into two groups, depending on the clinical severity of the disease.[3] The non-severe group consisted of patients who did not require critical care and/or mechanical ventilation, and with O_2 saturation ≥92% at room air upon admission.[3] The severe group comprised patients requiring critical care and/or mechanical ventilation, and with O_2 saturation <92% at room air upon admission. This study was given a full review and approved by the institutional board review of The Medical City.

Analysis

The demographic data for each case, which included patient age, sex, co-morbid illness, and respiratory status were determined by frequency, percentage, median and interquartile range. The age of both groups was subjected to Mann-Whitney U test, while sex and co-morbid illnesses were subjected to Chi-square and Fisher-exact test. Laboratory data for both groups were determined by frequency, percentage, median and interquartile range and subjected to Mann-Whitney U and Chi-square test where appropriate.

RESULTS

We identified a total of 325 patients diagnosed with COVID-19. There were 154 (47.4%) women and 171 (52.6%) men with a median age of 62 years (range, 19 to 94 years). Two hundred twenty (67.7%) patients were classified as non-severe, and 105 (32.3%) patients were classified as severe. Among the severe cases, 69 (65.7%) ended in mortality, while 36 (34.2%) recovered. All 220 (67.6%) patients in the non-severe group recovered. Patient age was found to be significantly associated with disease severity ($p< 0.001$), while patient sex had no association ($p=0.068$) (Table 1).

All laboratory parameters, including Ct value, were found to be associated with disease severity ($p<0.001$). Lower Ct values of *ORF1ab* (median = 26.4) and *N* (median = 24.8) genes were seen in the severe group compared to the non-severe group. For hematologic parameters, the severe group showed increased neutrophils (81%) and decreased lymphocytes (8%) compared to the non-severe group, while platelet count remained within the normal range for both groups. Platelet count remained within range for both groups, however, there were generally lower counts in the severe group (median = 215, $p=0.001$). The coagulation parameter of PT/INR was found to be within the normal range for both groups, while an elevated D-dimer test was more commonly found in the severe group (n=58/105, 55.2%). Tests for liver enzymes showed elevation of AST/SGOT in the severe group compared to the non-severe group, while ALT/SGPT remained within range. Markers of inflammation – including LDH, ferritin, procalcitonin, and CRP – were all found to be more elevated in the severe group compared to the non-severe group. A tabulated summary of these findings is seen in Table 2.

Nearly all patients (n=311/325, 95.7%) had community-acquired pneumonia (CAP). The most common co-morbid illness was diabetes mellitus (n=93/325, 28.6%), followed by hypertension (n=89/325, 27.4%). There was also a variety of other illnesses which included conditions such as acute kidney injury (AKI) (n=20/325, 6.2%), acute respiratory distress syndrome (ARDS) (n=49/325, 15.1%), septic shock (n=36/325, 11.1%), and others (n=116/325, 35.7%) – heart failure, myocardial infarction, acute gastroenteritis, asthma, chronic obstructive pulmonary disease, Alzheimer's disease, acquired immunodeficiency syndrome, bipolar disorder, breast cancer, lung cancer, pancreatic cancer, multiple myeloma, T-cell Acute Lymphoblastic Lymphoma, cerebrovascular disease, hypothyroidism, multinodular goiter, intracerebral hemorrhage, pulmonary tuberculosis, urinary tract infection, acute pyelonephritis, and

Table 1. Patient demographic characteristics

Characteristics	All patients (n = 325)	Non-severe (n = 220)	Severe (n = 105)	*p*
Age, years	62 [12]	59 [20]	68 [22]	<0.001[#]
Sex				
Male	154 (47.4%)	111 (50.5%)	43 (41.0%)	0.068[$]
Female	171 (52.6%)	109 (49.5%)	62 (59.0%)	

Median [Interquartile Range]; Frequency (%); [#]Mann-Whitney U-test, [$]Chi-square test

Table 2. Ct value and pertinent laboratory parameters among patients with COVID-19

Characteristics	Reference range	All patients (n = 325)	Non-severe (n = 220)	Severe (n = 105)	*p*
ORF1ab Gene	≤40	29.0 [8.4]	30.1 [7.7]	26.4 [6.6]	<0.001#
N Gene	≤40	27.2 [8.2]	28.3 [7.8]	24.8 [6.8]	<0.001#
Neutrophil	56–66%	76 [17]	72 [17]	81 [11]	<0.001#
Lymphocyte	22–40%	18 [17]	22 [17]	9 [11]	<0.001#
Platelet count	140–440 x 10^9/L	245 [122]	258 [118]	215 [121]	<0.001#
D-dimer	0.0–0.50 ug/mL	0.7 [1.2]	0.6 [0.7]	1.3 [2.2]	<0.001#
Not elevated	<0.50 ug/mL	79 (24.3%)	61 (27.7%)	18 (17.1%)	<0.001$
Elevated	≥0.50 ug/mL	126 (38.8%)	68 (30.9%)	58 (55.2%)	
PT/INR	0.8–1.1	1.0 [0.1]	1.0 [0.1]	1.0 [0.1]	0.003#
LDH	120–246 U/L	369 [218]	326 [164]	487 [268]	<0.001#
Ferritin	17.9–464.00 ng/mL	792.5 [1,241.0]	647.0 [907.0]	1,350.0 [1,868.0]	<0.001#
Procalcitonin	0.0–0.50 ng/mL	0.1 [0.5]	0.1 [0.2]	0.5 [3.0]	<0.001#
Not elevated	<0.50 ng/mL	223 (68.6%)	172 (78.2%)	51 (48.6%)	
Elevated	≥0.50 ng/mL	77 (23.7%)	25 (11.4%)	52 (49.5%)	<0.001$
CRP	1.00–3.00 mg/L	86.6 [117.5]	68.5 [109.7]	137.9 [175.3]	<0.001#
AST/SGOT	17.0–59.0 U/L	61 [52]	53 [43]	75 [70]	<0.001#
ALT/SGPT	0.0–50.0 U/L	46 [49]	45 [47]	48 [57]	<0.341#

median [interquartile range]; frequency (%); #Mann-Whitney U-test; $Chi-square test

Table 3. Co-morbid illnesses present in patients with COVID-19

Co-morbid illness	All patients (n = 325)	Non-severe (n = 220)	Severe (n = 105)	*p*
Diabetes mellitus	93 (28.6%)	60 (27.3%)	33 (31.4%)	0.259$
Hypertension	89 (27.4%)	53 (24.1%)	36 (34.3%)	0.037$
ARDS	49 (15.1%)	5 (2.3%)	44 (41.9%)	<0.001*
Septic shock	36 (11.1%)	1 (0.5%)	35 (33.3%)	<0.001*
AKI	20 (6.2%)	6 (2.7%)	14 (13.3%)	<0.001*
CAP - High risk	104 (32.0%)	15 (6.8%)	89 (84.8%)	<0.001*
CAP - Moderate risk	202 (62.2%)	186 (84.5%)	16 (15.2%)	
CAP - Low Risk	5 (1.5%)	5 (2.3%)	0 (0.0%)	
Other illnesses	116 (35.7%)	86 (39.1%)	29 (27.6%)	0.028*

Frequency (%); $Chi-square test; *Fisher-exact test

Table 4. Respiratory status of patients with COVID-19

Respiratory status	All patients (n = 325)	Non-severe (n = 220)	Severe (n = 105)
Room air (ward)	99 (30.5%)	99 (45.0%)	0 (0.0%)
Nasal cannula	114 (35.1%)	114 (51.8%)	0 (0.0%)
Face mask	7 (2.2%)	7 (3.2%)	0 (0.0%)
Room air (ICU)	15 (4.6%)	0 (0.0%)	15 (14.3%)
Intubated	86 (26.5%)	0 (0.0%)	86 (81.9%)
Tracheostomy	4 (1.2%)	0 (0.0%)	4 (3.8%)

Frequency (%); $Chi-square test

myocarditis. There was also a higher percentage of patients with co-morbid illness in the severe group (Table 3). The presence of co-morbid illness, except for diabetes mellitus, was found to be significantly associated with disease severity (Table 3).

The majority of patients in the non-severe group were placed on oxygen support via nasal cannula (n=114/325, 35.1%), or tolerated room air (n=99/325, 30.5%), while majority of patients in the severe group were intubated (n=86/325, 26.5%) (Table 4).

DISCUSSION

This study finds that Ct values are lower in the severe group compared to the non-severe group for both *ORF1ab* and *N* genes (Table 2, Figure 1). In addition, the severe group had worse respiratory status, since these patients needed ICU admission, intubation, or tracheostomy (Table 4). These patients were in the older age group (median = 68 years, interquartile range [IQR] = 22), and had worse clinical outcomes consisting of High-Risk CAP, ARDS, AKI, and septic shock (Table 3). This appears to be consistent with data reported from a metanalytic study by Rao et al.[7] Their findings state that lower Ct values (median = 34.79) were seen in patients who died compared to those who survived (median = 37.43); lower Ct values were also present in patients who had severe disease progression (Ct value = 24); and that lower Ct values were associated with an increased risk of mortality.[7] In addition, Ct values below 30 were found to be associated with a higher risk of severe disease and hospitalization in comparison to those patients with Ct-value higher than 30.[25,26] However, it should be noted that the studies described in the meta-analysis involved mostly hospitalized adult patients, and may be subject to a population bias.

Another meta-analysis however, found no association between COVID-19 disease and Ct value.[25,27] Their findings were more variable, wherein the Ct value was either increased or decreased among hospitalized patients; did not differ among patient groups with differing disease severity; and the Ct values were not associated with risk of hospitalization.[25,27] The reason for these findings may be

due to several factors, majority of which are pre-analytic. These factors include timing of taking patients' sample, the adequacy of the swab material, and the type of sample included in each study.[7,25-27] There are also factors inherent to the PCR process itself which can affect the Ct value. These factors include the presence of inhibitors within the patient's swab sample, the PCR test kit used, the kit reagents, and the efficiency of RT-PCR procedure.[8,26-28] Furthermore, though Ct value is used as a surrogate marker for viral load in the absence of a viral culture, they may not have a linear relationship due to the aforementioned pre-analytic and analytic factors described.[6,26] Majority of RT-PCR kits available locally for COVID-19 are qualitative in nature, as complex procedures are required to standardize quantitative PCR tests.[9] Due to the heterogenous findings on the correlation of Ct values with clinical outcomes and laboratory parameters among patients with COVID-19, we maintain that qualitative reporting of Ct value is sufficient for rendering a diagnosis. The reporting of Ct value may be considered on a case-to-case basis, with particular emphasis that clinicians should correlate these values with their overall clinical and laboratory assessment per patient.[9,26,27]

For the pertinent laboratory parameters, abnormal values were present in both groups of patients, however the inflammatory biomarkers, coagulation studies, hematologic markers and liver enzymes were more elevated in the severe group (Table 2). Specifically for hematologic markers, patients with severe COVID-19 disease had higher neutrophil count (median = 81%, IQR = 11) and lower lymphocyte count (median = 9%, IQR = 11) (Figure 2). Findings of neutrophil and lymphocyte counts are similar to other studies, and it is suggested that lymphocyte counts below 0.8 x 10^9/L and neutrophil counts higher than 3.5 x 10^9/L reflect a poor clinical outcome and are associated with COVID-19 disease severity.[14,22] Besides the differential count, the determination of Neutrophil-Lymphocyte ratio (NLR) is of interest to clinicians, as a higher NLR was found to be associated with worse clinical outcomes in inflammatory conditions, some cancers, and as a predictor of cardiovascular mortality.[22,29,30] It is proposed that infection with the SARS-CoV-2 virus triggers activation of the innate immune system, manifested by neutrophilia and elevated acute phase reactants, which was found in our study.[29,30] Therefore in the local setting, it is recommended to determine complete blood counts of COVID-19 patients, in order to compute the NLR for prognostication and clinical management.

The coagulation markers routinely tested in COVID-19 patients in the local setting include PT/INR and D-dimer, and in the current study this was found to be associated with severe disease (Table 2, Figure 5).[3] Platelet counts were lower in the severe group ($p<0.001$) (Table 2, Figure 3), the median D-dimer value was higher (median = 1.3 ug/mL, IQR = 2.2), while PT/INR values were similar between groups (median = 1.0, IQR = 0.1). This is similar to the findings in a meta-analysis, wherein patients with severe disease exhibit thrombocytopenia, significant elevation in D-dimer, but with variable findings of PT/INR.[14,22,31] In particular, the abnormalities of coagulation have been associated with those COVID-19 patients who are in the ICU setting.[14,32] These findings point to an underlying coagulopathy caused by the virus, whose pathophysiology

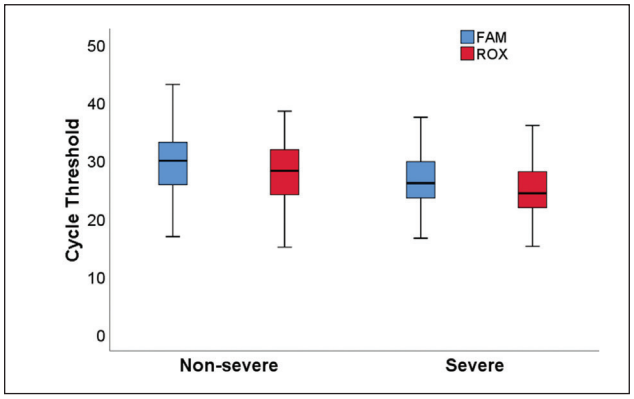

Figure 1. Cycle threshold values in non-severe and severe groups of patients with COVID-19.

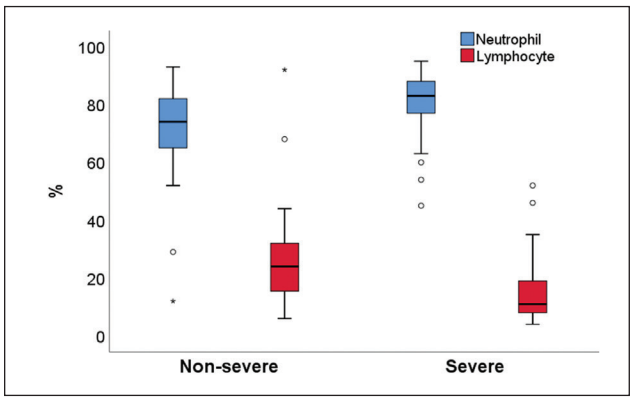

Figure 2. Neutrophil and Lymphocyte counts of non-severe and severe patients with COVID-19.

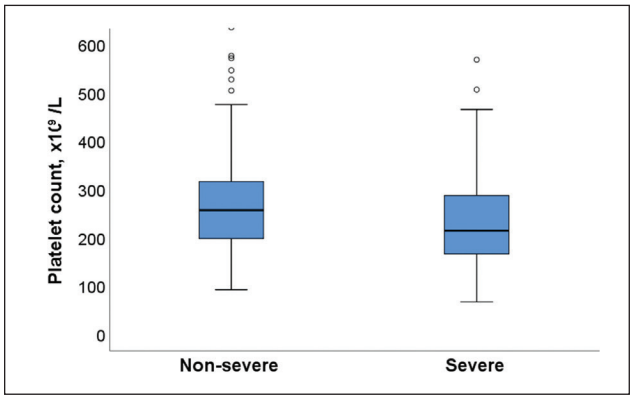

Figure 3. Platelet counts among non-severe and severe patients with COVID-19.

is not yet well understood.[22,30,33] It is proposed that one of the mechanisms of coagulopathy is viral infection of the vascular endothelial cells, causing injury and activation of the fibrinolytic system with microthrombi formation particularly in the pulmonary circulation.[32] In addition, D-dimer is associated with various critical illnesses such as venous thromboembolism (VTE), disseminated intravascular coagulopathy (DIC); and elevated levels are widely recognized as a poor prognostic marker.[6,22,33] Therefore, it is recommended to test for these coagulation parameters in the local setting.

Several acute phase reactants (APRs) are recommended locally to be tested in patients with COVID-19, which include CRP, Ferritin, LDH and Procalcitonin.[3] This study finds that all patients with COVID-19 exhibit elevated levels of these reactants, which is expected due to the inflammatory response against the virus (Figures 4 and 5).[6,34] Higher levels of these APRs were found to be associated with more severe disease (Table 2), which is similarly reported in other studies.[7,14,22,34,35] A study by Sayit et al., report the following findings: that a CRP value higher than 20.42 mg/L can predict the severity of COVID-19 disease; elevated LDH is a strong predictor of lung injury in COVID-19 as it is released from the cytoplasm of necrotic cells; Ferritin levels increase due to stimulation by proinflammatory cytokines and leakage from damaged cells; and that elevated Procalcitonin levels are related to a 5-fold higher risk of severe COVID-19.[34] It is of benefit to clinicians to monitor these APRs as they correlate with disease severity and prognosis.

In the local setting, it is recommended to test for liver function markers as COVID-19 may affect this organ, hence the inclusion of AST and ALT.[3] The current study finds that AST is associated with more severe disease ($p<0.001$), while no association is seen in ALT ($p= 0.341$) (Table 2, Figure 6). The significant increase in AST compared to ALT may be due to the presence of the mitochondrial isoenzyme of AST, which has a long half-life (87 hours), and may also be due to the fact that AST is not limited to the liver and can be found in other organs such as the heart.[6,14,26] In addition, it is widely recognized that the SARS-CoV-2 virus may cause damage to other organs which express the ACE2 receptor, as this is the point of attachment of the virus.[14,26] This receptor is expressed in organs such as the lungs, liver, heart, and kidneys, hence the work-up for organ involvement in COVID-19 disease should not be limited to liver function tests. Other studies report that patients with severe COVID-19 disease present with abnormal cardiac markers such as elevated troponins, and with abnormalities in kidney function like elevated creatinine.[14,22,34] Therefore there is merit for clinicians to expand their laboratory work-up where appropriate in order to facilitate care for COVID-19 patients with multiple organ involvement.

For the co-morbid illnesses, the most common in this population was diabetes mellitus (n=93/325, 28.6%) followed by hypertension (n=89/325, 27.4%) (Table 3). This is the opposite of the profile of other studies, which found hypertension (32.5% of patients) to be more common than diabetes mellitus (24.10% of patients).[26,35] In the current study, the median age of involved Filipino patients is 62 (IQR = 12), which is older than the population in a similar study by Yormaz et al., which was performed in Turkey (average age = 56.3 years).[35] Older age and co-morbid conditions (particularly hypertension and diabetes) are found to be associated with more severe disease (Table 1, Table 3), and are predisposed to prolonged viral shedding.[26] Lower Ct values have been found in older patients with COVID-19, and this has been attributed to their slower immune response due to immunosenesence.[6,26] In addition, older patients tend to have more frequent co-morbid illnesses, therefore the age factor should be taken into consideration as it is correlated with more severe outcomes.[26,35]

Limitations of the study include the retrospective design, which analyzed the laboratory parameters and Ct values of patients at a single point in time. Trends or changes of Ct value and laboratory parameters were not determined. Another limitation was not accounting for the timing of nasopharyngeal swabs for SARS-CoV-2 and timing of blood collection in relation to the day of illness in the study. This may act as a confounder because drawing any kind of association between Ct values and other lab test results requires that specimen collection for all of them be done at the same time. As mentioned earlier, a factor that could

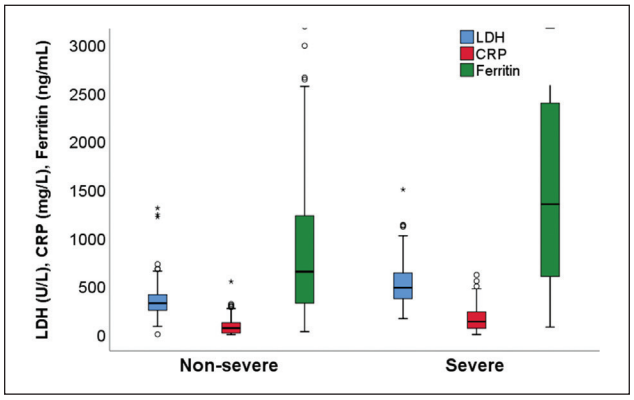

Figure 4. Acute Phase Reactants among non-severe and severe patients with COVID-19.

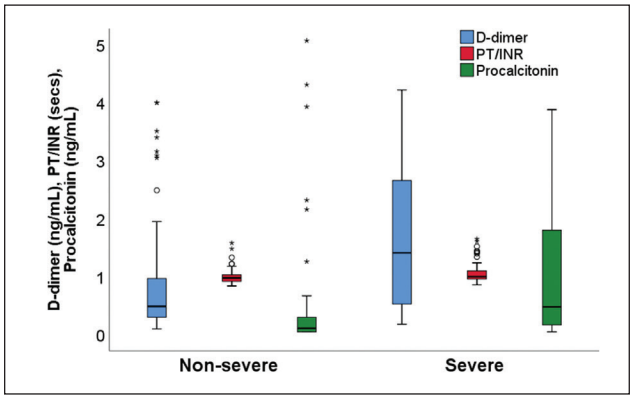

Figure 5. Coagulation markers and procalcitonin among non-severe and severe patients with COVID-19.

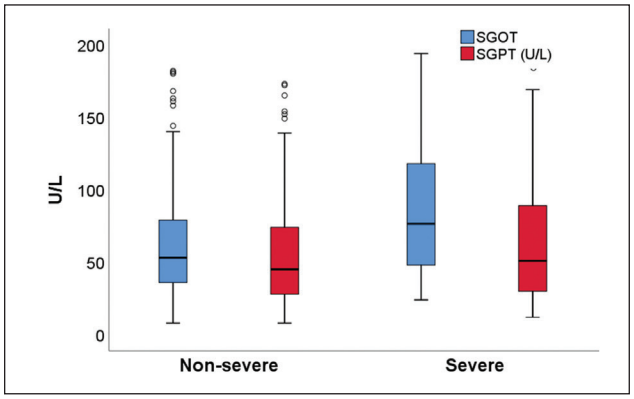

Figure 6. Liver enzymes among non-severe and severe patients with COVID-19.

influence Ct values is the time interval between collection and processing (age of the specimen). A long interval before processing could affect the stability of the viral RNA and thus lead to high Ct values or false negatives.[12] Factors that could affect this time interval include stat requests from doctors, availing of tests for non-clinical purposes (e.g., travel, employment), and requests for home swabbing, among others. When such factors are not accounted for, it is possible for them to behave as confounders, as these various situations are related to both the Ct values and the clinical picture of the patient. Interpretations of the laboratory values must be taken into the proper clinical context. The study was carried out during the period when variants of the SARS-CoV-2 virus were not yet identified. Different variants of SARS-CoV-2 can also confound the outcomes, as they could have different properties from each other in terms of infectivity, replication, immunogenicity, pathophysiology or severity. The study is also limited to the symptomatic adult population who were hospitalized and does not represent the entire spectrum of the disease. Asymptomatic patients were not included as the majority had no laboratory workup performed. Multivariate analysis to account for confounding factors and potential biases was not done as this was beyond the scope of the study.

The authors recommend that further investigation should be undertaken in the manner of pursuing correlational studies, or prospective cohort studies, taking into account the timing of specimen collection with the correlation of clinical parameters. This is of interest, especially in the light of newly developing medical interventions, mass population vaccinations, and the recognition of Post COVID-19 syndrome.[36] Pursuing studies to include non-hospitalized patients may be of benefit to adequately represent how COVID-19 affects the local community, and to guide policies on containment and spread of the virus. There is also limited data on COVID-19 in pediatric patients, and looking into this population may be of interest to facilitate pediatric patient care. Investigation of various biomarkers of organ involvement and how these change in COVID-19 patients may also provide further insight into the pathophysiology of the infection and aid patient management and therapeutic decision-making.

CONCLUSION

Laboratory biomarkers such as neutrophil and lymphocyte counts, LDH, ferritin, procalcitonin, CRP, D-dimer, PT/INR, and AST are associated with severe COVID-19 disease. Lower Ct-values, older age, and the presence of co-morbid illness are also associated with severe COVID-19 disease. The qualitative reporting of SARS-CoV-2 results as positive or negative is sufficient for diagnosis of the disease, and with the currently available data, the reporting of Ct values may be considered on a case-to-case basis by clinicians to aid in patient management decisions. The Ct values should be interpreted with caution, given the multiple pre-analytic and analytic factors which may affect the result. Instead, the patient's overall clinical profile, laboratory biomarkers and Ct value should be taken as a whole to guide therapeutic decision-making.

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STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

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REFERENCES

1. Ludwig S, Zarbock A. Coronaviruses and SARS-CoV-2: a brief overview. Anesth Analg. 2020;131(1):93-6. PMID: 32243297. PMCID: PMC7173023. https://doi.org/10.1213/ANE.0000000000004845.
2. World Health Organization. Philippines Coronavirus Disease 2019 (COVID-19) Situation Report # 83. Transmission stage assessment; 2021.
3. Philippine Society for Microbiology and Infectious Diseases. Interim guidance on the clinical management of adult patients with suspected or confirmed COVID-19 infection (version 3.1); 2020. https://www.psmid.org/interim-management-guidelines-for-covid-19-version-3-1/.
4. World Health Organization. WHO in-house assays for 2019-novel coronavirus (2019-nCoV) real-time rRT-PCR; 2020. https://www.who.int/docs/default-source/coronaviruse/whoinhouseassays.pdf. Accessed February 28, 2022.
5. Wang H, Li X, Li T, et al. The genetic sequence, origin, and diagnosis of SARS-CoV-2. 2020;39(9):1629-35. PMID: 32333222. PMCID: PMC7180649. https://doi.org/10.1007/s10096-020-03899-4.
6. McPherson RA, Pincus MR. Henry's clinical diagnosis and management by laboratory methods. 24th ed. Elsevier Inc.; 2021.
7. Rao SN, Manissero D, Steele VR, Pareja J. A systematic review of the clinical utility of cycle threshold values in the context of COVID-19. Infect Dis Ther. 2020;9(3):573-86. PMID: 32725536. PMCID: PMC7386165. https://doi.org/10.1007/s40121-020-00324-3.
8. Tom MR, Mina MJ. To interpret the SARS-CoV-2 Test, consider the cycle threshold value. 2020;71(16):2252-4. PMID: 32435816. PMCID: PMC7314112. https://doi.org/10.1093/cid/ciaa619.
9. AACC recommendation for reporting SARS-CoV-2 cycle threshold (CT) values. 2021. www.aacc.org/-/media/Files/Science-and-Practice/Statements/

Recommendationsreporting-SARSCoV2-CT-values.pdf.
10. IDSA and AMP joint statement on the use of SARS-CoV-2 PCR cycle threshold (Ct) values for clinical decision-making. 2021. https://www.idsociety.org/globalassets/idsa/public-health/covid-19/idsa-amp-statement.pdf.
11. APHL. Ct values: what they are and how they can be used. 2020. https://www.aphl.org/programs/preparedness/Crisis-Management/Documents/APHL-COVID19-Ct-Values.pdf.
12. Punchoo R, Bhoora S, Bangalee A. Laboratory considerations for reporting cycle threshold value in COVID-19. EJIFCC. 2022;33(2):80–93. PMID: 36313906. PMCID: PMC9562486.
13. Tsang NNY, So HC, Ng KY, Cowling BJ, Leung GB, Ip DKM. Diagnostic performance of different sampling approaches for SARS-CoV-2 RT-PCR testing: a systematic review and meta-analysis. Lancet Infect Dis. 2021;21(9):1233-45. PMID: 33857405. PMCID: PMC8041361. https://doi.org/10.1016/S1473-3099(21)00146-8.
14. Moutchia J, Pokharel P, Kerri A, et al. Clinical laboratory parameters associated with severe or critical novel coronavirus disease 2019 (COVID-19): a systematic review and meta-analysis. PLoS One. 2020;15(10):e0239802. PMID: 33002041. PMCID: PMC7529271. https://doi.org/10.1371/journal.pone.0239802.
15. He X, Lau EHY, Wu P, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med. 2020;26(5):672–5. PMID: 32296168. https://doi.org/10.1038/s41591-020-0869-5.
16. Hall SM, Landaverde L, Gill CJ. Comparison of anterior nares CT values in asymptomatic and symptomatic individuals diagnosed with SARS-CoV-2 in a university screening program. PLoS ONE. 2022;17(7):e0270694. PMID: 35830378. PMCID: PMC9278773. https://doi.org/10.1371/journal.pone.0270694.
17. Long H, Zhao J, Zeng HL, et al. Prolonged viral shedding of SARS-CoV-2 and related factors in symptomatic COVID-19 patients: a prospective study. BMC Infect Dis. 2021;21(1):1282. PMID: 34961470. PMCID: PMC8711078. https://doi.org/10.1186/s12879-021-07002-w.
18. Yan D, Liu XY, Zhu YN, et al. Factors associated with prolonged viral shedding and impact of lopinavir/ritonavir treatment in hospitalised non-critically ill patients with SARS-CoV-2 infection. Eur Resp J. 2020;56(1): 2000799. PMID: 32430428. PMCID: PMC7241115. https://doi.org/10.1183/13993003.00799-2020.
19. Zhu H, Fu L, Jin Y, et al. Clinical features of COVID-19 convalescent patients with re-positive nucleic acid detection. J Clin Lab Anal. 2020;34(7):e23392. PMID: 32506726. PMCID: PMC7300578. https://doi.org/10.1002/jcla.23392.
20. Samprathi M, Jayashree M. Biomarkers in COVID-19: an up-to-date review. Front Pediatr. 2021;8:607647. PMID: 33859967. PMCID: PMC8042162. https://doi.org/10.3389/fped.2020.607647.
21. Ghahramani S, Tabrizi R, Lankarani KB, et al. Laboratory features of severe vs . non - severe COVID - 19 patients in Asian populations : a systematic review and meta-analysis. Eur J Med Res. 2020;25(1):30. PMID: 32746929. PMCID: PMC7396942. https://doi.org/10.1186/s40001-020-00432-3.
22. Pourbagheri-Sigaroodi A, Bashash D, Fateh F, Abolghasemi H. Laboratory findings in COVID-19 diagnosis and prognosis. Clin Chim Acta. 2020;510:475-82. PMID: 32798514. PMCID: PMC7426219. https://doi.org/10.1016/j.cca.2020.08.019.
23. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001;69(3):89-95. PMID: 11240971. https://doi.org/10.1067/mcp.2001.113989.
24. Sansure Biotech Inc. Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluoresence Probing). 2020. https://www.sansureglobal.com/product/s3102e-sc2/.
25. Shah VP, Farah WH, Hill JC, et al. Association between SARS-CoV-2 Cycle threshold values and clinical outcomes in patients with COVID-19: a systematic review and meta-analysis. Open Forum Infect Dis. 2021;8(9):ofab453. PMID: 34584900. PMCID: PMC8465328. https://doi.org/10.1093/ofid/ofab453.
26. Rabaan AA, Tirupathi R, Sule AA, Aldali J, Mutair A Al. Viral dynamics and real-time RT-PCR Ct values correlation with disease severity in COVID-19. Diagnostics (Basel). 2021;11(6):1091. PMID: 34203738. PMCID: PMC8232180. https://doi.org/10.3390/diagnostics11061091.
27. Camargo JF, Lin RY, Komanduri K V. Lack of correlation between the SARS-CoV-2 cycle threshold (Ct) value and clinical outcomes in patients with COVID-19. J Med Virol. 2021;93(10):6059-62. PMID: 34196409. PMCID: PMC8427039. https://doi.org/10.1002/jmv.27171.
28. Joynt GM, Wu WK. Understanding COVID-19: what does viral RNA load really mean? Lancet Infect Dis. 2020;20(6):635-6. PMID: 32224308. PMCID: PMC7118539. https://doi.org/10.1016/S1473-3099(20)30237-1.
29. Ruiz SJ, Ventura PS, García MM, et al. Prognostic implications of neutrophil-lymphocyte ratio in COVID-19. Eur J Clin Invest. 2021;51(1):e13404. PMID: 32918295. https://doi.org/10.1111/eci.13404.
30. Tatum D, Taghavi S, Houghton A, Stover J, Toraih E, Duchesne J. Neutrophil-to-lymphocyte ratio and outcomes in Louisiana COVID-19 patients. Shock. 54)5):652-8. PMID: 32554992. PMCID: PMC7326320. https://doi.org/10.1097/SHK.0000000000001585.
31. Pablo J, Hinojosa R, Rodriguez Y, et al. Association between cycle threshold (C t) values and clinical and laboratory data in inpatients with COVID-19 and asymptomatic health workers. J Med Virol. 2021;93(10):5969-76. PMID: 34196423. PMCID: PMC8427125. https://doi.org/10.1002/jmv.27170.
32. Martín-Rojas RM, Pérez-Rus G, Delgado-Pinos VE, et al. COVID-19 coagulopathy: an in-depth analysis of the coagulation system. Eur J Haematol. 2020;105(6):741-50. PMID: 32749010. PMCID: PMC7436538. https://doi.org/10.1111/ejh.13501.
33. Ichkawa Y, Wada H, Ezaki M, et al. Elevated D-dimer levels predict a poor outcome in critically ill patients. Clin

Appl Thromb Hemost. 2020;26:1076029620973084. PMID: 33347372. PMCID: PMC7755937. https://doi.org/10.1177/1076029620973084.
34. Sayit AT, Elmali M, Gedikli O. Relationship between acute phase reactants and prognosis in patients with or without COVID-19 pneumonia. Rev Inst Med Trop Sao Paulo. 2021;63:e51.
35. Yormaz B, Ergun D, Tulek B, et al. The evaluation of prognostic value of acute phase reactants in the COVID-19. Bratisl Lek Listy. 2020;121(9): 628-33. PMID: 32990010. https://doi.org/10.4149/BLL_2020_103.
36. Vanichkachorn G, Newcomb R, Cowl CT, et al. Post COVID-19 syndrome (Long Haul Syndrome): description of a multidisciplinary clinic at the Mayo Clinic and characteristics of the initial patient cohort. Mayo Clin Proc. 2021;96)7):1782-91. PMID: 34218857. PMCID: PMC8112396. https://doi.org/10.1016/j.mayocp.2021.04.024