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Original Research Article

Correlation of Hematological Parameters to Cycle Threshold Value of Real Time Reverse Transcriptase-Polymerase Chain Reaction Positive COVID-19 Patients Admitted in Intensive Care Unit: Study from a Tertiary Care Center in Eastern India

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Abstract

Introduction: Coronavirus disease 2019 (COVID-19) is a pandemic caused by the novel coronavirus SARS-CoV2, causing an enormous strain on the already burdened healthcare systems. The clinical course of COVID-19 is variable; those with a poor prognosis tend to develop severe viral pneumonia requiring ventilator support and intensive care unit (ICU) admission.

Aim & Objectives: The aim of this study is to correlate the Cycle Threshold (Ct) score of RT-PCR reaction with different biomarkers like White cell count (WCC), Neutrophils%, Lymphocytes%, Monocytes%, Neutrophil-Lymphocyte ratio(NLR) , Lymphocyte – Monocyte ratio (LMR), Platelet count, Prothrombin time(PT), Interleukin 6(IL-6), C-Reactive protein (CRP), Blood sugar level(BSL) .Thus enabling if a low Ct score can help early identification of patients at a high risk to progress to a severe disease.

Method: A prospective analytical study conducted at a tertiary care hospital, included 114 severe COVID-19 positive patients, admitted in ICU. The medical history, comorbidities, clinical findings, and laboratory data of each patient were obtained with data analyzed to identify and correlate significant laboratory parameters leading to the severe outcome.

Results: Total 114 patients were studied**.** The mean age of the study population was 59 years with a male predominance. Significant positive correlation of Ct values was seen with Total WBC counts (p=0.004), Neutrophil % (p=0.001), NLR (p<0.001), IL-6 (p=0.010), Procalcitonin (p=0.015) and D-dimer (p=0.041). Significant negative correlation of Ct value with Lymphocyte % (p=0.001) and monocyte % (p<0.001). And no significant correlation was seen with Age, Gender, LMR, CRP, Platelet counts, Prothrombin time and Blood Sugar levels.

Conclusion: It is known that biomarkers help in identifying the disease severity and mortality and help in proper diagnosis and patient treatment. Ct scores can be used as a surrogate marker of disease severity, although further studies are required to validate the same.

Keywords: Biomarkers, Interleukin-6, Procalcitonin, Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR), SARS- COV2.

Introduction

In December 2019, Wuhan City in China became the epicenter of unexplained cases of pneumonia and in January 2020, Chinese scientists labeled it as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). But its name was changed by the World Health Organization in February 2020 to Coronavirus disease 2019 (COVID-19) [1].

Coronaviruses (CoV) are a large family of viruses that cause diseases in a broad clinical spectrum; from mild common flu infection to Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV-2). Studies have estimated that while 30-60% of COVID-19 cases are asymptomatic or mildly symptomatic, 5% of symptomatic cases are critically ill [2].

Clinical or demographic risk factors for severe disease include older age, male sex, and chronic health conditions, especially diabetes mellitus, cardiovascular disease, immunosuppression and obesity [1]. Infected patients usually present with any of the following symptoms like high temperature (>37.3°C), cough, myalgia, headache, diarrhea, dyspnea and in some cases, acute respiratory distress syndrome (ARDS), acute cardiac injury or secondary infection [1].

These clinical symptoms can be easily interpreted with the use of biological markers (biomarkers). They provide objective values throughout the progression of the disease helping us in categorizing the patients into mild, moderate or severe criteria for early interventions [3].

Apart from the clinical symptoms and chest computed tomography (CT) findings, a large number of COVID-19 confirmed patients showed laboratory fluctuations including complete blood count (CBC) variables, cardiac, coagulation parameters, renal, liver function tests and inflammation-related factors.

These biomarkers give clinicians a tool to triage the patients and predict prognosis and mortality. In this article we aim to explore the correlation of these biomarkers with Ct score to ultimately analyze if Ct score can also be taken as a surrogate marker of disease severity. The biomarkers which we reviewed in our study include white cell count (WCC), Neutrophil%, Lymphocyte%, Neutrophil-Lymphocyte ratio (NLR), Lymphocyte-Monocyte ratio (LMR), Monocytes, Platelet count, D-dimer, Prothrombin time (PT), C-reactive protein (CRP), Interleukin-6 (IL-6), Procalcitonin (PCT) and blood sugar level (BSL). To the best of our knowledge this study is the first from this region and will further our understanding of management of future epidemic waves in a more efficient and diligent manner.

Materials and Methods

The study was a prospective analytical study for a period of 3 months (from April to June 2021) at tertiary care hospital.

114 Patients (COVID- 19 RT- PCR positive) who were admitted in ICU were selected for the study. The criteria for ICU admission was based on detailed clinical history including presenting complaints like fever, dyspnea, respiratory distress any of the following criteria like: Respiratory rate>30/min, Spo2<93 at rest and PaO2/FiO2<300mmHg

All cases were diagnosed as COVID-19 based on RT-PCR tests of nasopharyngeal and oropharyngeal samples. THE RT PCR test was conducted using TRUPCR VIRAL RNA Extraction Kit, TRUPCR SARS CoV-2 RT qPCR Kit, Kilpest, India as per the manufacturer instructions. RT-

PCR tests were conducted using ALTA Real Time PCR System RT48 Instrument.

The Envelope (E) gene and RNA dependent RNA polymerase (RdRp) Ct value was reported and used in this study. The tested specimen was considered positive for SARS-CoV-2 for the cycle threshold (Ct) value less than or equal to 35 for E gene and RdRp or either only RdRp. The positive and negative controls consisted of viral RNA plasmid and sterile nuclease-free water, respectively. Ct values > 35 were considered negative.

The following biomarkers were analyzed on admission:- white cell count (WCC), Neutrophil%, Lymphocyte%, Neutrophil-Lymphocyte ratio (NLR), Lymphocyte-Monocyte ratio (LMR), Monocytes, Platelet count were studied by five part Mindray BC-5150 along with manual slide confirmation. PT was measured by manual coagulometer Hemostar XF 2.0, Tulip Diagnostics P Ltd. D-dimer, BSL and CRP were analyzed by Semi automated Analyzer Erba Chem7, Transasia Biomedical. PCT was measured by Getein 8000 Quantitative Immunoassay Analyzer and Interleukin 6(IL-6) was analyzed by I Chroma II, Boditech biotechnology.

Exclusion Criteria: Non COVID cases, cases with mild COVID-19 symptoms not admitted in ICU were excluded.

All tests were carried out in accordance with relevant guidelines and regulations, good laboratory practices and informed consent obtained from all participants.

The findings were recorded on case proforma which was entered in Microsoft Excel 2010. Different statistical analysis was performed using R software version 4.0.2. The one-sample Kolmogorov – Smirnov test was employed to determine whether the data sets differed from a normal distribution or not. Normally distributed data was analyzed using parametric tests and non - normally distributed data was analyzed by using non parametric tests. Descriptive statistics were calculated for qualitative and categorical variables. Graphical representation of the variable was shown to understand the results clearly and to measure the association for categorical dataset which was analyzed using Chi-Square test. Independent T-test or student t-test was applied to measure the mean difference between two groups. Correlation was estimated to measure the strength of relationship between two or more quantitative variables.

If p value <0.05, it was considered as statistically significant and if p-value>0.05, then it was statistically insignificant.

Results

Out of the total 114 patients studied –

The most common age group was 59 years (range from 27-85 years)

The Ct screening value ranged from 18.4 to 35.0. Mean being 25.7 ±4.43.

The Ct confirmatory value ranged from 19.2 to 36.3. Mean being 25.9 ±4.51.

Descriptive statistics of the Severity parameters analyzed are given in Table 1.

Table 1: Descriptive Statistics.

Descriptive Statistics	Minimum	Maximum	Mean	Std. Deviation
Age	27.0	85.0	59.6	13.9
Ct Screening	18.4	35.0	25.7	4.4
Ct Confirmatory	19.2	36.3	25.9	4.5
WCC (/cumm)	1670.0	27000.0	10687.3	4926.5
Neutrophils (%)	52.0	96.0	83.1	11.4

Lymphocytes (%)	2.0	36.0	12.0	9.8
NLR	1.0	48.0	13.2	10.6
LMR	0.3	10.0	3.1	2.1
CRP(mg/L)	0.8	5438.0	95.7	505.5
Monocyte%	0.7	9.0	3.9	2.1
Platelet (lakh/cumm)	0.7	3.7	1.8	0.6
IL-6(pg/mL)	1.0	113.5	13.7	19.7
D-dimer (ng/ml)	0.0	32.6	1.5	4.2
PT (seconds)	10.5	72.5	13.9	6.0
PCT (ng/ml)	0.0	1.8	0.5	0.4
BSL (mg/dl)	5.1	495.7	185.4	98.9

WCC: – White cell Count, NLR: - Neutrophil-Lymphocyte ratio, LMR: - Lymphocyte Monocyte ratio, CRP:-C -Reactive protein, IL-6:- Interleukin-6, PT:- Prothrombin time, PCT:- Procalcitonin, BSL:- Blood sugar level.

Ct values showed a significant positive correlation with WCC (p=0.004), Neutrophil % (p=0.001), NLR (p<0.001), IL-6 levels (p=0.010), PCT levels (p=0.015) and D-dimer levels (p=0.041).
Ct values showed a Significant negative correlation with Lymphocyte % (p=0.001) and Monocyte % (p<0.001) Ct values did not show any significant correlation with Age, Gender, LMR, CRP levels, Platelet counts, PT and BSL. Correlations of Biomarkers with Ct confirmatory are given in Table 2.

Table 2: Correlation of Biomarkers with Ct confirmatory.

Variable Name	r value	p-value
Age (years)	-0.083	.388
WCC (cells/cumm)	0.274	**.004**
Neutrophils %	0.318	**.001**
Lymphocytes %	-0.299	**.001**
NLR	0.385	**.000**
LMR	-0.165	.084
CRP (mg/L)	0.100	.297
Monocyte %	-0.332	**.000**
Platelet (lakh/cumm)	0.027	.776
IL-6 (pg/mL)	0.243	**.010**
D-dimer (ng/ml)	0.196	**.041**
PT (seconds)	-0.009	.929
PCT (ng/ml)	0.297	**.015**
BSL (mg/dl)	0.025	.797

WCC: – White cell Count, NLR: - Neutrophil-Lymphocyte ratio, LMR: - Lymphocyte Monocyte ratio, CRP:-C -Reactive protein, IL-6:- Interleukin-6, PT:- Prothrombin time, PCT:- Procalcitonin, BSL:- Blood sugar level.

Discussion

COVID-19 is a rapidly spreading pandemic which has increased the burden on health care system [1]. The heterogeneous disease course of COVID-19 is unpredictable with most patients experiencing mild self-limiting symptoms. However up to 30% require hospitalization, and up to 17% of these require intensive care support for acute respiratory distress syndrome (ARDS), hyper inflammation and multiorgan failure [2]. Real Time Reverse Transcription Polymerase Chain Reaction (Real Time RT-PCR) is the gold standard test for detection of SARS-CoV-2, enabling us in early detection of viral genome in clinical samples. A positive test enables the clinicians to isolate the patient and prevent spread of the disease [4]. High viral load was found to be an independent predictor of disease severity and mortality in few studies, and in susceptible individuals such as elderly, patients with co-existing medical illness such as diabetes, heart diseases and immunosuppressed [5].

The Ct value of a RT-PCR reaction is the number of cycles at which fluorescence of the PCR product is detectable over and above the background signal. Theoretically, the Ct value is inversely proportional to the amount of genetic material (RNA) in the starting sample and lower Ct values generally correlate with high viral load [6]. In addition, to its correlation with viral load, it also has a strong correlation with multiple hematological and biochemical markers [4,7].

High viral load is also associated with elevated levels of IL-6 and C reactive protein contributing to a hyper-inflammatory state and severe infection [5]. However, Ct values might be affected by pre-analytic, analytic, and post-analytical variables. [4]. Therefore, understanding the interpretation of Ct values and other influential factors could play a crucial role in interpreting viral load and disease severity [5].

This study has analyzed correlation of Ct score with biomarkers for their usefulness in COVID-19 disease stratification. We have demonstrated that RT-PCR report with Ct values at time of admission correlate with various biomarkers like WCC, Neutrophils%, Lymphocyte%, Monocyte%, NLR, LMR, Platelets, CRP, IL-6, D-Dimer, PT, PCT and BSL which in turn are indicative of different aspects of COVID-19 severity hospital stay and mortality.

Studies of Biswas M et al [8] show evidence that male patients, age ≥50 years, or with comorbidities are significantly associated with increased risk of mortality in COVID- 19. In our study the mean age of patient is 59 years.

It was stated that male patients may have higher expression of angiotensin-converting enzyme 2 (ACE2) receptors, which may be regulated by male sex hormones rendering them to more risk for SARS-CoV-2 infection and poor clinical outcomes as well [8]. Concurrent with the above statement the predominant population in the current study was of males (65%).

In our study the mean white cell count was raised in the study population. This data was similar to the other studies [1,3,9,10]. The Ct score correlated significantly with the WCC (p value = 0.004) thereby indicating that Ct score can be a surrogate marker for disease severity.

Studies show that lymphocytes play an important role, lymphopenia is noted in severely ill patients.[1,9,11] In our study the COVID-19 patients showed increased neutrophil count (range- 52% to 96%, mean 83.1 %) and decreased lymphocyte counts (range 2-36%, mean 12%) during the severe phase of COVID-19 infection. The Ct score correlated significantly with the Neutrophil % (p value = 0.025) and negatively correlated with Lymphocyte %

(p value = 0.016) thereby indicating that Ct score can also be a surrogate marker for disease severity.

Neutrophil-to-lymphocyte ratio (NLR) is one of the most well-established inflammatory markers that reflect systemic inflammatory response to stress thus reflecting the disease severity. Therefore, this biomarker is an indirect indication of the body's stress level due to the severity of the disease. In our study NLR was raised which showed an enhanced inflammatory response which was associated with disease severity [12,13,14]. The Ct score correlated significantly with the NLR (p value = 0.004) thereby indicating that Ct score can also be a surrogate marker for disease severity. Monocytes in most of the cases were decreased whereas basophils and eosinophil were not significant. [11, 15] In our study the Ct scores correlated significantly with Monocyte % (p value = 0.000).

The coagulation indicators include D-Dimer, PT and platelet counts which play an important role in COVID-19. Rising levels of D-dimer indicates the activation of coagulation and fibrinolysis. It is raised more in non-survivors or severe COVID 19 patients than non-severe patients [1] Tang et al Zhou et al mentioned that D-dimer levels >1 μg/mL can help clinicians in identifying patients with poor prognosis at earlier stage. D-dimer levels are much higher in those requiring ICU admission. [1,9,12]. Thus D-dimer plays an important role in triage during admission, hospital stay and to differentiate severe vs non-severe patients thus helping in treating the patients. It had a positive correlation with Ct value in our study (p value = 0.041).

Prothrombin time (PT) and Activated Plasma Thromboplastin time are exogenous and endogenous coagulating system factors, which can be used for early diagnosis of Disseminated Intravascular Coagulation commonly seen in COVID 19 patients [6]. The dynamic coagulating process in patients with COVID-19 is likely due to the hyper coagulating state followed by the activation of fibrinolysis [6,12,14]. Our study shows a significant positive correlation of D-dimer with Ct score (p value = 0.041).

Inflammatory biomarker of COVID 19 includes Interleukin **-6** levels which have a positive correlation with the severity of disease. Cytokine release syndrome (CRS) is an exaggerated immune response involving an overwhelming release of pro-inflammatory mediators [9]. This mechanism underlies several pathological processes including Acute respiratory distress syndrome (ARDS) seen in COVID- 19 [1]. It is hypothesized that SARS-CoV-2 first binds to the alveolar epithelial cells and then the virus triggers our innate immune system and the adaptive immune system, leading to the release of cytokines, including IL-6, which is a pleiotropic cytokine which is the core in regulating immunological and inflammatory responses [9].

Studies have revealed that levels of IL-6, rise sharply in severe manifestations of COVID-19[1]. Our study shows mean IL-6 concentrations were 2.9-fold higher in patients with severe COVID-19 with a significant p value (p value = 0.10) of correlation with Ct score. Numerous studies [12,14,16] have showed positive correlation of IL-6 with disease severity and mortality. Thus, a helpful marker to assess and treat accordingly.

Procalcitonin (PCT) is a peptide precursor of the hormone calcitonin. During bacterial infection, the production and release of PCT into the circulation from extra thyroidal sources is increased that is maintained by increased levels of IL-6, IL-1β, and TNF-α whereas the increased concentration of interferon-γ during viral infection is negatively impacting the synthesis of PCT. This is why the level of PCT remains within the normal range in the majority of the patients with non-severe COVID-19 and increased value in

severe COVID-19 may indicate secondary bacterial infection [9]. Lippi et al. along with other studies showed that increased PCT levels are associated with a 5-fold higher risk of severe COVID-19 [2, 9, 10]. In our study the PCT levels significantly correlated with Ct score (p value = 0.015).

Another biomarker is C- reactive protein (CRP), an acute phase reactant used clinically as a biomarker for various inflammatory conditions. A rise in CRP levels is associated with an increase in disease severity. CRP is said to be an effective biomarker to predict the progression of COVID-19 infection [9, 11, 16]. CRP levels do not significantly correlate with Ct score (p value >0.05).

In various studies [13, 14, 17, 18] it is seen that diabetic COVID-19 patients had higher levels of leukocytes, neutrophils, lower levels of lymphocytes and eosinophil, and higher levels of inflammatory markers such as CRP, ferritin, D-dimer and FDP, than non-severe patients [17] . The pathogenesis behind is it due to defect in innate immunity affecting phagocytosis, neutrophil chemotaxis, and cell-mediated immunity. [18]. Other studies like , Kornum et al. suggested that subjects with type 1 diabetes had a 4.4-fold increased risk of a pneumonia-related hospitalization, and subjects with type 2 diabetes displayed a 1.2-fold increased risk of a pneumonia-related hospitalization compared with those without diabetes [18]. Blood sugar levels (BSL) did not correlate significantly with the Ct score (p value >0.05).

Conclusion:

To sum up our study the Ct values showed significant positive correlation with WCC, Neutrophil%, NLR, IL-6 levels, PCT and D-Dimer levels. COVID-19 is a heterogeneous disease spectrum with manifestations varying with age and presence of co-morbidities. It is a known fact that biomarkers play an important role in early suspicion, diagnosis, monitoring, and recognition of complications, management of patients. This study shows that Ct score is a fairly good parameter to indicate the chances that a patient might land up as a severe case. Thus, patients with RT-PCR reports showing a lower Ct score should be kept under supervision and close monitoring. Further multicentric studies are required to determine the role of Ct score as an indicator of disease severity in a qualitative RT-PCR test.

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