ARE BASIC INFLAMMATION MARKERS SUFFICIENT FOR PREDICTING COVID 19 INFECTION

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ABSTRACT

Introduction*: Although Covid 19 is a systemic disease lung involvement causes the main clinical outcomes. As chest ct, inflammatory markers were other important parameters that provide information about the disease course.*

Methods*: We enrolled 150 in-hospital patients between 02 April 2020-03 May 2020. The severity of disease classified as mild, moderate, severe, and critically ill. Chest CT of the patients assessed and classified for the degree of parenchyma involvement and noted as following: No involvement (0%), Minimal involvement (1-25%) Mild involvement (26-50%), Moderate involvement (51-75%) and severe involvement 76-100%) The relationship between inflammatory markers, chest ct and severity of disease statistically evaluated.*

Results*: There were no statistical differences between the Chest CT involvement and INR, Ast, Alt, Creatinine, Uric acid, Platelet, White blood cell and procalcitonin levels (all p > 0.05). A positive correlation was found between Ct involvement and fibrinogen, D-dimer, sedimentation rate, ferritin, NLR, Crp (r = 0.478, p = <0.001; r =0.311, p = <0.001; r =0.455, p = <0.001; r= 0.369, p = <0.001; r= 0.584, p = <0.001, respectively) Roc analysis revealed that CRP>9.5 mg/L predicted the severe disease with 96% sensitivity and 97.5% specificity.*

Conclusion*: Evaluating Covid 19 infection with easily accessible markers is important. Crp, D-dimer, Ferritin and NLR stands out to as easily accessible, cheap, and reliable markers in determining the prognosis of disease.*

Keywords: *Covid 19 infection, markers.*

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Introduction

Coranavirus disease 2019 (Covid 19) infected over 12 million and caused over 500000 death up to July 2020[(1)]. The main route of the transmission is respiratory droplets and patients usually present with respiratory symptoms like dry cough, fever, fatigue and sometimes atypical presentations also with loss of taste and smell. Although Covid 19 is a systemic disease lung involvement causes the main clinical outcomes. Chest computerized tomography (CT) played a great role both in diagnosis and the management of the patients. Inflammatory markers were other important parameters that provide information about the disease course[(2,3)]. C-reactive protein (Crp), Procalcitonin (Pct), Ferritin are the most commonly used and most easily reached markers.

In previous studies inflammatory markers have been shown to provide useful information about the course of disease. However the correlation of inflammatory marker levels both with chest CT findings and disease course was not considered yet. Here we aimed to focus on correlation of chest CT findings and disease severity with inflammatory marker levels.

Method

This is a retrospective single center study performed in Gebze Government Hospital. We enrolled 150 in-hospital patient between 02 April 2020-03 May 2020. The study was approved by the local ethics committee and Ministry of Health Covid 19 Science Board.

Covid 19 diagnosed according to WHO guidance. Nasopharyngeal swap samples were used for real-time reverse transcriptase polymerase chain reaction (rt-PCR) tests. Patients older than 18 years and confirmed as Covid 19 infection by PCR testing were enrolled in the study.

Data collection

Patients demographicfeatures, symptoms and signs, co morbidities, laboratory tests and chest computed tomography (CT) scans were evaluated. All data were collected from the electronic hospital information system. The medical records of the patients were reviewed by an expert investigator. Peripheral venous blood samples were measured at the biochemical laboratory of Gebze Government hospital following standard operative procedures. The routine blood tests (including white blood cell count [WBC], leukocyte subtypes (neutrophil, lymphocyte, eosinophil, basophil), hemoglobin count and platelet count) were measured with multi-function automatic blood analyzer. Biochemical parameters were measured with the ARCHITCT ci16200 automatic biochemistry analyzer (Abbott Laboratories, Illinois, United States)CRP and serum ferritin were measured using latex enhanced immunoturbidimetry (Cobas 8000; Roche)

Assessment of Disease Severity

Mild disease was defined as absence of dyspnea and patients with fever, malaise, cough, upper respiratory symptoms, and/or less common features of COVID-19. Patients who developed dyspnea without hypoxia was defines as moderate disease. Severe COVID-19 was defined as the existence of dyspnea, blood oxygen saturation ≤ 94% on air room and need for oxygenation or ventilatory support.(4)Critical COVID-19 was defined as the existence of respiratory failure, septic shock and/or multiple organ dysfunctions[5].

Chest CT of the patients assessed and classified for the degree of parenchymainvolvementand noted as following: No involvement (0%), Minimal involvement (1-25%) Mild involvement (26-50%), Moderate involvement (51-75%) and severe involvement 76-100%)[6].

Statistical Analysis

Statistical analyses were performed using SPSS for Windows version 24.0 (SPSS, Chicago, IL, USA). The one-sample Kolmogorov-Smirnov test was used to verify the normality of data distributions. Results are expressed as numbers, percentages; median, minimum and maximum. One-way ANOVA with a post hoc Bonferroni and Kruskall–Wallis tests were used in normally and nonnormally distributed continuous data, respectively. Pearson correlation analysis is used to find any relationship between the CT involvement and fibrinogen, D-dimer, sedimentation rate, ferritin, NLR, Crp levels. ROC-curve analysis was performed to find the cut-off values of serum Ferritin, Crp, D-dimer and NLR levels. Values of $P < 0.05$ were considered statistically significant for all results.

Results

Table 1,2 summarizes the demographic, clinical and biochemical characteristics of all patient. There were no statistical differences between the Chest CT involvement andINR,Ast,Alt, Creatinine, Uric acid,Platelet, White blood cell and procalcitonin levels (all p> 0.05). A positive correlation was found between Ct involvement and fibrinogen, D-dimer, sedimentation rate, ferritin, NLR,Crp (r =0.478, p =<0.001; r =0.311, p = <0.001; r =0.455, p = <0.001; r= 0.369, p = <0.001; r= 0.584, p = <0.001, respectively);

	Mild (n:15)	Moderate (n:56)	Severe (n:73)	Critically ill (n:6)	Total (n:150)
Age	40 (18-60)	47 (23-79)	63 (20-97)	70,5 (56-80)	54 (mean)
Male	11	37 (66.0%)	41 (56.0%)	4 (66%)	93 (62.0%)
Female	4	19 (33.0%)	32 (43.0%)	2 (33%)	57 (38.0%)
Co morbidity	4 (26.0%)	37 (50.6%)	69 (94.6%)	4 (66%)	119 (79.3%)
Hypertension	2 (9.5%)	7 (12.5%)	8 (1.0%)	0	17 (11.3%)
Diabetes	2 (9.5%)	9 (16.0%)	10(13.6%)	0	21 (14.0%)
Chronic kidney disease	0	1 (0.1%)	6 (0.8%)	0	7 (4.0%)
Cardiovascular disease	0	12 (21.4%)	24 (32.8%)	3 (50%)	39 (26.0%)
Respiratory disease	0	7 (12.5%)	13 (17.8%)	1 (16%)	21 (14.0%)
Chronic liver disease	0	1 (0.1%)	2 (0.2%)	0	3 (2.0%)
Solid tumor	0	0	4 (0.5%)	0	4 (2.6%)
Hematologic malignancy	0	0	1 (0.1%)	0	1 (0.6%)
Rheumatic disease	0	0	1 (0.1%)	0	1 (0.6%)
No co morbidity	11(73%)	19 (40%)	5 (7%)	2 (33%)	37 (24.6%)

Table 1: Demographic characteristics of patients with COVID-19.

In ROC curve analysis, ferritin levels above 82.35 showed 64% sensitivity and 56% specificity for predicting severe involvement in Chest CT [area under the curve (AUC) = 0.628; 95% confidence interval (CI) = 0.531–0.729]and levels above 82.35 showed 67% sensitivity and 65% specificity for predicting severe disease course [area under the curve (AUC) = 0.705; 95% confidence interval (CI) = 0.623–0.787]. Crp levels above 14.50 showed 80% sensitivity and 70% specificity for predicting severe involvement in Chest CT [area under the curve (AUC) = 0.792; 95% confidence interval (CI) = 0.719–0.865] and levels above 9.5 showed 96% sensitivity and 97.5% specificity for predicting severe disease course[area under the curve (AUC) =

0.974; 95% confidence interval (CI) = 0.955–0.993]. NLR levels above 2.75 showed 72% sensitivity and 60% specificity for predicting severe involvement in Chest CT [area under the curve (AUC) = 0.717; 95% confidence interval (CI) = 0.628–0.806] and levels above 2.90 showed 64% sensitivity and 68% specificity for predicting severe disease course [area under the curve (AUC) = 0.694; 95% confidence interval (CI) = 0.610–0.778]. In addition, ferritin levels above 358.35 showed 67% sensitivity and 99% specificity for predicting the death rates [area under the curve (AUC) = 0.744; 95% confidence interval (CI) = 0.562–0.926].

Laboratory	Mild (n:15)	Moderate (n:56)	Severe (n:73)	Critically ill (n:6)
Leukocyte count, 10^3 cells/L	7.78 (3.9-13.6)	7.81 (2.5-18.4)	9.8 (2.6-34.3)	6.82 (4.4-10.8)
Neutrophil count, 10^3 cells/L	3.99 (2.4-9.07)	4.18 (1.4312.12)	6.25 (0.00-78.0)	3.96 (2.26-6.91)
Lymphocyte count, 10^3 cells/L	2.04 (0.64-3.88)	1.78 (0.55-7.44)	1.49 (0.00-5.49)	1.56 (0.7-4.28)
Monocytes count, 10^3cells/L	0.56 (0.36-1.46)	0.69 (0.18-5.00)	0.72 (0.00-5.20)	0.57 (0.18-1.17)
Hemoglobin level, g/L	12.7 (8.3-16.10)	13.8 (7.4-16.7)	12.0 (6.4-16.7)	13.9 (11.2-15.8)
Platelet count, 10^3 cells/L	288.0 (133.0-385.0)	250.0 (53.0-546.0)	223.0 (13.8-491.0)	188.5 (120.0-329.0)
Fibrinogen, g/L	245.0(198.0-580.0)	356.0(231-619)	455.0(233.0-749.0)	463.0(397.0-588.0)
D-dimer, mg/L	0.37(0.14-0.58)	0.47(0.08-6.29)	1.17(0.08-25.06)	2.1 80.35-3.46)
C-reactive protein level, mg/L	4.53 (1.0-9.0)	8.0 (2-17)	23.0 (9-54)	30.0 (28-39)
ESR, mm/60 min	12.0(10.0-12.0)	14.0(10.0-54.0)	23.0(11.0-101.0	17.5(11.0-36.0)
Serum ferritin ng/ml	90.6 (7.0-370.6)	63.5 (4.0-1086.0)	132.2(6.9-1834.0)	161.4 (32.5-1650)
Creatinine level, mg/dl	0.8(0.58-1.38)	0.83(0.38-2.64)	0.93(0.55-3.2)	0.81(0.58-1.49)
Urea level, mg/dl	12.0 (7.0-43.0)	12.0(5.0-63.0)	17.0(6.0-156.0)	12.5(7.0-23.0)
Procalcitonin ng/ml	0.04 (0.01-0.41)	0.03 (0.00-1.93)	0.09 (0.0-60.96)	0.06(0.01-0.56)
Neutrophil/ Lymphocyte	1.93(0.89-4.07)	2.41(0.7-14.55)	4.05(0.0-24.26)	2.49(1.19-6.60)

Table 2: Laboratory findings of patients with COVID-19.

Discussion

Covid 19 pandemic have been the world's nightmare for about 8 months. Although there are improvements in treatment strategies there is still no specific treatment option. Therefore patient evaluation and predicting the prognosis is important. Inflammation markers and chest ct scans provided important information about the disease course. But how reliable are these markers in predicting the course of disease. In this study we sought for an answer to this question.

We compared the inflammation markers and ct findings at the time of admission with disease severity.

In our study extensivity of involvement in chest ct correlated well with disease severity and inflammatory markers. Patients with more than 50% involvement in chest ct at the admission had a severe disease course. Common CT features of COVID-19 are ground-glass opacities (GGOs) and consolidation with or without vascular enlargement, interlobular septal thickening, and air bronchogram. Lesions are more likely to have peripheral distribution and bilateral involvement. But these findings not only specific for Covid 19 infection may also be present in other viral infections like Adenovirus[7]. Our aim was to determine the prognosis, so we planned our study based on width of the involvement not type of the involvement. Similar findings were highlighted by Francone et al. In their study Ct score was significantly higher in severe than mild stage patients and also Ct score correlated well with the inflammatory markers as we found in our study[8].

CRP is an acute phase inflammatory protein produced by the liver that may be elevated in several conditions, such as inflammation, cardiovascular disease and infection[9]. In previous studies done by L wang CRP levels positively correlated with the diameter of lung lesions and disease severity course[10]. Zhou et al showed that markedly elevated Crp, Ferritin, PCT levels were related with the severe disease and poor prognosis[11]. In a meta-analysis with a total of 5350 patients CRP levels > 10 mg/L was associated with poor outcome and severe disease and had a 51% sensitivity, 88% specificity[12]. In our study we found a positive correlation with CT score and disease severity. As the severity of the disease increased, CRP levels elevated clearly and markedly. In our study Roc analysis revealed that CRP>9.5 mg/L predicted the severe disease with 96% sensitivity and 97.5% specificity. We found very close value of CRP level indicating the severe disease course when compared with this meta analysis but sensitivity and specificity were much higher in our study. CRP is an easily accessible and practical marker and shows a clear correlation with the course of disease. Therefore we consider CRP as a reliable parameter.

Ferritin is a major intracellular iron storage protein but also an acute phase reactant. High circulating ferritin levels may not only reflect an acute phase response, also play a critical role in inflammation with the induction of the expression of dif-

ferent inflammatory mediators, including IL-1β[13]. Eleveted ferritin levels are associated with severe and critical Covid 19 disease. However some studies reported that ferritin levels increase in all patients[14]. In our study we found significantly increased levels of ferritin only in severe and critical group. We also examined the relationship between ferritin levels and Chest CT involvement and significantly elevations was only in patients with more than 75% Chest Ct involvement. The increase in ferritin levels was not completely correlated with disease severity but the levels abruptly increased in severe group. In our study Roc analysis revealed that ferritin levels above 358.35 showed 67% sensitivity and 99% specificity for predicting the death rates. Qin C et al found this level 350.86 and in meta analysis including three study that focus on ferritin levels the average level of ferritin was found 398.8 . These results were approximately similar with our study[15,16].

According to these results abruptly increases of ferritin levels is related with severe disease course and poor outcome. Therefore we believe that monitoring the ferritin level will provide useful information about the disease course.

Covid 19 infection could induce the dysfunction of the hemostatic system, leading to a hypercoagulable state[17]. Lung pathology dissections has shown occlusion and micro-thrombosis formation in pulmonary small vessels of patients critically ill patients with COVID-19[18]. Hence D-dimer levels elevates in severe Covid 19 infection. In a study of Yu et al D-dimer levels were elevated in severe disease course and correlated well with hsCRP level. In follow up levels were decreased with treatment. But d- dimer levels had a limited predictive value for thrombosis[19].

This result emphasizes thatinflammation is one of the causes of coagulation activation. In our study D-dimer levels were also elevated in severe and critical patients. When we compare the Chest CT involvement with D-dimer levels. D- dimer levels were elevated as the CT involvement increases. This finding also supported positive correlation between inflammatory markers and CT involvement.

The neutrophil to lymphocyte ratio (NLR), easily calculated from a routinely blood test by dividing absolute neutrophil count by absolute lymphocyte count, has been reported of having great value in indicating a patient's overall inflammatory status[20]. Severe COVID-19 infection tended to have higher NLR. Qin C et al, Mo P, Liu Y et al reported that severe cases of COVID-19 were likely to have higher neutrophil count but lower lymphocyte count compared with non-severe patients, thus the NLR tended to be higher in severe infection patients[21,22,23]. We reached the same findings in our study. NLR is a rapid, easily available, useful prognostic factor in the early screening of severe and critical illness in patients with confirmed COVID-19 infection.

Conclusion

Covid 19 continues to spread rapidly and brings heavy costs for governments. Therefore, it is important to evaluate the disease with easily accessible markers. Crp, D-dimer, Ferritin and NLR stands out to as easily accessible, cheap, and reliable markers in determining the prognosis of disease.

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