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Pathology

LYMPHOCYTE RATIO, LEUCOCYTE COUNT AND D-DIMER IN EARLY PROGNOSTICATION OF COVID-19 PATIENTS: A PROSPECTIVE STUDY

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ABSTRACT COVID-19 was declared as a global pandemic by the year 2020 , caused by the severe acute respiratory syndrome coronavirus – 2 (SARS-CoV-2) strain which claimed the lives of millions globally. Hospitals have been burdened with the admissions of the disease and the categorization of patients as per clinical severity for the treatment. Early detection of severity prognostic biomarkers will aid in the decreased morbidity and mortality of diseased patients undergoing treatment. Novel biomarkers like neutrophil to lymphocyte ratio, D-dimer and Leukocyte count are useful for the categorization of these patients and to assess their disease severity. These markers individually are used as inflammatory markers and markers of thrombotic events. The assessment of utility of these biomarkers would be beneficial for screening , better management , and reduction of serious complications for pandemics like COVID-19 . All age groups are affected by the pandemic , co-morbidities in addition increase the severity of the disease.

KEYWORDS : COVID-19, categorization, biomarkers, pandemic.

INTRODUCTION

In March 2020, the World Health Organization (WHO) declared COVID-19 disease as a global pandemic. It will not be wrong to say that the pandemic, caused by the severe acute respiratory syndrome coronavirus – 2 (SARS-CoV-2) strain, wreaked havoc, leading to more than 700 million confirmed cases and more than 7 million deaths globally, as of 2024.[1] As the COVID-19 pandemic spread across all parts of the world, the hospitals and intensive care units (ICUs) were overwhelmed with a heavy load of severe cases, and with limited knowledge of the disease, managing the severe cases was a challenge for all clinicians. In India, this was an even bigger challenge with the massive population completely exhausting the hospital beds, and the lack of clarity on managing severe COVID pneumonia adding to the difficulty in managing these cases. The scientific community is in urgent need for reliable biomarkers related to COVID-19 disease progression, to stratify high risk patients and predict the prognosis. The rapid disease spread necessitates the immediate categorization of patients into risk groups following diagnosis, to ensure optimal resource allocation. Novel biomarkers are needed to identify patients who will suffer rapid disease progression to severe complications and death. Effective biomarkers would be helpful for screening, clinical management, and prevention of serious complications.

Amongst the many biomarkers being evaluated to predict early prognosis of COVID-19, some like D-dimer, neutrophil-to-lymphocyte ratio (NLR) as well as leukocyte count have attracted great attention globally. D-Dimer is a specific degradation product that is produced in hydrolysis of fibrin.[2] It may reflect the effects of infection on coagulation in infectious diseases. Some studies reported increase in D-Dimer levels in patients with pneumonia, has an indication of the blood hypercoagulable state and the presence of thrombosis.[3,4] D-Dimer of critically ill patients with COVID-19 was significantly increased, with frequent clotting disorders and microthrombotic formation in peripheral blood vessels.[5] Neutrophil-lymphocyte ratio is a marker of systemic inflammation that has a quick and simple operation, and predicts prognosis in various pathological conditions.[6,7] Recently, NLR was found to have greater prognostic power than traditional infection markers, such as CRP, white blood cell count and neutrophil count, in community-acquired pneumonia.[8,9] At the early stage of COVID-19, the total number of leukocytes in peripheral blood is normal or decreases, while the lymphocyte count decreases. Systemic inflammatory response syndrome (SIRS) is the systemic inflammatory response to a variety of severe clinical insults. Infection including virus infection is an important inducer of systemic inflammatory response.[10] Published data have indicated that the confirmed COVID-19 patients with increased leukocyte count had significantly higher level of systemic inflammation response, which at least partially related to the development of critical illness, with a high admission to an ICU and a high mortality rate.[11]

A literature search revealed that there are very few studies which have evaluated the role of D-dimer, NLR and leukocyte count in COVID-19 cases, as early prognostic indicators. Most of these studies published are from China or western studies, and neither of the studies have evaluated these biomarkers together in the Indian population. Hence, it was decided to evaluate the utility of D-dimer, NLR and leukocyte count in COVID-19 cases, as early prognostic indicators at an Indian tertiary care teaching hospital. The assessment of utility of these biomarkers would be helpful for easier screening, clinical management, and prevention of serious complications for pandemics like COVID-19 in future.

METHODS

Study participants

A prospective, observational study was conducted at Department of Pathology, Saifee Hospital in Mumbai City of India. The study was initiated only after institutional ethics committee permission was obtained, and data was collected between November 2020 to May 2022. All adult patients diagnosed with COVID-19 by RT-PCR and admitted to hospital, tested for complete blood count and D-dimer on admission with Covid-19 at the time of admission, peak of rise in counts of parameters and post admission were enrolled. The exclusion criteria included the following: patients who did not undergo COVID-19 RTPCR testing or whose RTPCR reports are unavailable, patients with primary co-infection, pregnant females, patients whose subsequent data is not available for consecutive values at admission, peak of values of parameters or post admission in MRD records. Written informed consent was taken from all enrolled cases in study.

Parameters assessment of enrolled patients

Demographic details of the enrolled patients (age, gender, comorbidities) were noted.Patient laboratory records of neutrophil-lymphocyte ratio (NLR) and leucocyte count from Complete Blood Counts (CBC) records and values of D-dimer were taken at the time of admission and consequent values at the peak of rise in counts of parameters and post admission as well. NLR was calculated by analyzing differential leucocyte count in CBC tests. D-dimer was calculated from Biomerieux Vidas by the Enzyme-Linked Fluorescent Assay (ELFA) method. The CBC and D-dimer assessment tests were noted at the time of admission, peak of rise in counts of parameters and post admission (3 serial counts).

Statistical analysis

There was a total of 20651 total admissions (COVID-19 + non-COVID-19) during the study period, and of these, 2891 patients were having COVID-19 infection. Considering this value, the incidence of COVID-19 infection was 13.9%. The minimum sample size obtained was 183, at a confidence interval of 95% and power of the study of 80%.

Data analysis was done with the help of statistical software Graphpad InStat.v3.0 Quantitative data (age etc.) was presented with the help of Mean and Standard deviation. Descriptive statistics were evaluated using numbers and percentages. The comparison between continuous variables in mild, moderate and severe COVID-19 subgroups was done using unpaired t test. The intragroup change in the continuous variables in mild, moderate and severe COVID-19 subgroups was done using repeated measures ANOVA. The sensitivity, specificity, AUC and cut-off levels were evaluated for the first WBC count, neutrophil, lymphocyte and D-dimer levels to predict severe COVID

using receiver operating characteristic (ROC) curve. P value of less than 0.05 was considered to be statistically significant.

RESULTS

Demographic and baseline characteristics

A total of 189 patients were enrolled in the study. The mean age with SD was 62.63+15.72 years, with an age range of 24 to 91 years. Majority of enrolled cases were males (n=119, 62.96%). The age distribution analysis revealed the majority being between 51-70 years of age (n=85, 44.97%) followed by 71-90 years' age group (n=62, 32.8%) and 31-50 years (n=32, 16.93%). The commonest comorbidity noted in the study was hypertension (46.56%), followed by Diabetes mellitus (DM) (38.1%) and ischemic heart disease (IHD) (15.34%). 26.46% of cases had no comorbidities in the study.

Most of the enrolled cases in study were mild COVID cases (n=70, 37.04%), followed by moderate COVID (n=60, 31.75%) and severe COVID (n=59, 31.22%). Mean age was significantly higher (p<0.05) in the severe COVID group versus mean age in both mild-COVID and moderate COVID groups. The gender distribution in all groups (mild, moderate, and severe COVID) were statistically comparable (p>0.05) (Table 1).

Table 1: Comparison of demographic details between Mild, moderate and Severe COVID cases

Parameter assessed	*Mild COVID (n=70)*	*Moderate COVID (n=60)*	*Severe COVID (n=59)*
Age comparison			
Mean age (years)	59 ± 17.69	62.4 ± 16.77	68.02 ± 10.64
P value	**<0.01* considered significant**		
Gender distribution			
Number of males	41 (58.57%)	42 (70%)	36 (61.01%)
Number of females	29 (41.43%)	18 (30%)	23 (38.98%)
P value	0.37, considered NOT signficant		

P<0.05 considered significant by one-way ANOVA test (age) and chi-square test (gender) WBC, NLR and D-dimer between Mild, moderate and Severe COVID cases at various time points (Table 2) Over period of observation, the mean WBC count in mild COVID, moderate COVID and severe COVID groups were noted to significantly increasing (p<0.05). However, over serial time points, mean NLR in the COVID groups were noted to statistically comparable (p>0.05).

Mean D-dimer levels in mild COVID and moderate COVID were noted to statistically decrease serially over time-points of observation (p<0.05), while the reduction was non-significant in severe COVID group (p>0.05).

Table 2: Comparison of WBC count and NLR between Mild, moderate, and severe COVID cases at various time points

Type of COVID cases	*1st observation (day 1)*	*2nd observation (day 2 or 3)*	*3rd observation (day 3-5)*	*P value^*
WBC count				
Mild COVID (n=70)	6895.57 ± 3137.7	9118.71 ± 3611.6	10426.6 ± 3278.7	**0.01^**
Moderate COVID (n=60)	7234.17 ± 3056.4	10511.3 ± 5064	11716.17 ± 6361.8	**0.01^**
Severe COVID (n=59)	9922.54 ± 5360.8	13722.4 ± 4455.2	14773.2 ± 5461.53	**0.01^**
P value*	**0.03***	**0.01***	**0.02***	
NLR				
Mild COVID (n=70)	4.44 ± 4.21	4.78 ± 2.85	5.23 ± 6.03	0.45
Moderate COVID (n=60)	5.77 ± 7.73	7.68 ± 9.06	5.66 ± 4.8	0.31
Severe COVID (n=59)	8.13 ± 7.06	10.96 ± 8.29	11.99 ± 14.75	0.28
P value*	**0.01***	**0.01***	**0.01***	
D-dimer				
Mild COVID (n=70)	1393.15 ± 1883.9	665.02 ± 616.18	566.47 ± 536.9	**<0.01^**
Moderate COVID (n=60)	2415.4 ± 9372.7	1209.2 ± 2337.6	877.72 ± 1215.1	**<0.01^**
Severe COVID (n=59)	4430.1 ± 12018.3	2308.13 ± 3119.7	2002.3 ± 7979.96	0.08
P value*	**0.01***	**0.01***	**<0.01***	

P<0.05 considered significant by one-way ANOVA test* and repeated measures ANOVA test^

On intergroup comparison, the mean WBC count, NLR and d-dimer levels were all noted to be significantly higher in severe COVID group versus other groups. (p<0.05).

AUC, Sensitivity, Specificity and Cut-off for First WBC count, NLR and D-dimer level observations to predict severe COVID:

At a cut-off of 6610 WBC cells/mm^3, the sensitivity by ROC curve was noted to be 72.88% (CI: 60.4%-82.56%), and the specificity was found to be 56.15% (CI: 47.57% to 64.39%) to predict severe COVID. The AUC for first WBC count observation to predict severe COVID was 0.68 (CI: 0.59-0.76) which was significant (p=0.0001*) (Figure 1).

At a cut-off of >5.02 NLR, the sensitivity by ROC curve was 64.41% (CI: 51.66%-75.4%), and the specificity was 67.69% (CI: 59.25% to 75.12%) to predict severe COVID. The AUC for first NLR finding to predict severe COVID was 0.67 (CI: 0.58-0.75) which was significant (p=0.001*) (Figure 2).

For first D-dimer assessment, at a cut-off of >861.5 ng/ml, the sensitivity by ROC curve was 67.8% (CI: 55.11%-78.3%), and the specificity was 64.62% (CI: 56.08% to 72.31%) to predict severe COVID. The AUC for first d-dimer observation to predict severe COVID was 0.69 (CI: 0.60-0.76) which was significant (p=0.001*) (Figure 3).

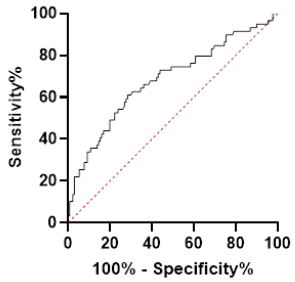

Figure 1: ROC Curve to evaluate the AUC, sensitivity, and specificity First WBC count observation to predict severe COVID.

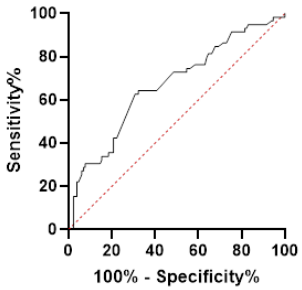

Figure 2: ROC Curve to evaluate the AUC, sensitivity, and specificity First NLR observation to predict severe COVID.

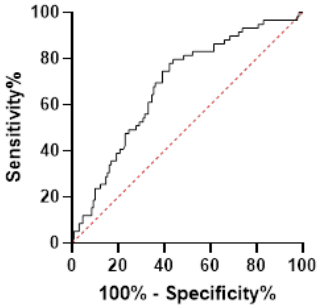

Figure 3: ROC Curve to evaluate the AUC, sensitivity, and specificity First D-dimer observation to predict severe COVID.

DISCUSSION

In our study, the utility of D-dimer, NLR and leukocyte count in COVID-19 cases, as early prognostic indicators was evaluated, at an Indian tertiary care teaching hospital. There was a high proportion of moderate to severe COVID cases overall, as many of the mild cases were prescribed isolation at home for the designated period (at least 7 days) and were managed by tele-consultation during the COVID waves. Mean age was noted to be significantly higher in the severe COVID group versus mean age in both mild-COVID and moderate COVID groups, in the study. There is an abundance of data published to prove that older age groups were affected with severe COVID more commonly, and this fact was reiterated in our present study as well. Adults over 65 years of age represent 80% of hospitalizations and have a 23-fold greater risk of death than those under 65. Comorbidities such as cardiovascular disease, diabetes, and obesity, which are common in the elderly, increase the chances of fatal disease. An abundance of recent data describing the pathology and molecular changes in COVID-19 patients points to both immunosenescence and inflammaging as major drivers of the high mortality rates in older patients.[12]

In our study, based on ROC findings, the sensitivity, specificity, and AUC for WBC, NLR and D-dimer were noted to be significant in predicting severe COVID-19. NLR was found to have greater prognostic power than traditional infection markers, such as CRP, white blood cell count and neutrophil count, in community-acquired pneumonia.[8,9] Just like our study finding, published scientific evidence from the foreign countries have also established that increased NLR may accurately govern the COVID-19 severity and can be utilized to recognize cases with severe disease to direct clinical decision-making. In a meta-analysis by Wang et al,[13] the potential of the neutrophil-lymphocyte ratio (NLR) as an indicator of severe versus non-severe COVID-19 cases was assessed. Thirty studies, including 5570 patients, were analyzed. Of these, 1603 and 3967 patients had severe and non-severe COVID-19, respectively. The overall sensitivity as well as specificity were 0.82 (95% confidence interval (CI), 0.77-0.87) and 0.77 (95% CI, 0.70-0.83), respectively. The AUC was 0.87 (95% CI, 0.84-0.90) implying that the NLR could accurately predict severe COVID-19 cases. In another study by Imran et al.,[14] NLR was established to be an independent risk factor for severe COVID-19 pneumonia in the heavy group (OR = 1.264, 95% CI: 1.046~1.526, P<0.05). The evaluated AUC using ROC for NLR was 0.831, with an optimal limit of 4.795, sensitivity of 0.83 and specificity of 0.75, that is highly indicative of NLR being a marker for the initial discovery of worsening severe COVID-19 infection. NLR can be utilized as an early warning signal for worsening severe COVID-19 infection and can deliver an objective basis for timely identification and treatment of severe COVID-19 pneumonia. The study of Imran et al. mentioned a NLR cut-off of 4.795, which was close to the 5.02 noted in our study. In study by Liu et al and Ye et al., the NLR cut-off predictive of severe COVID were noted to be 3.13 and 7.28 respectively and finding in our study (5.02) fits in this range.[15,16]

At the early stage of COVID-19, the total number of leukocytes in peripheral blood is normal or decreased. Systemic inflammatory response syndrome (SIRS) is the systemic inflammatory response to a variety of severe clinical insults. Infection including virus infection is an important inducer of systemic inflammatory response.[10] A study by Zhao et al. showed that the confirmed COVID-19 patients with increased leukocyte count were more likely to develop SIRS. Data have indicated that the confirmed COVID-19 patients with increased leukocyte count had significantly higher level of systemic inflammation response, which at least partially related to the development of critical illness, with a high admission to an ICU and a high mortality rate.[11] This can be one of the reasons why in the severe COVID-19 subset of patients in our present study, the WBC count was higher in comparison to the non-severe COVID subgroups.

In our study, a direct relationship was noted between D-dimer levels and severity of COVID-19. D-Dimer is a specific degradation product that is produced in hydrolysis of fibrin.[2] Some studies reported increase in D-Dimer levels in patients with pneumonia, has an indication of the blood hypercoagulable state and the presence of thrombosis.[3,4] Tang et al. demonstrated that cases who died from COVID-19 had significantly higher D-dimer levels and pro-thrombin time (PT) on admission in comparison to COVID-19 infection surviving cases.[17] Numerous studies also showcase a relationship between the trend of D-dimer levels as well as COVID-19 disease progression.[18,19] Initial studies equated the increase in D-dimer level with DIC, but ongoing work has indicated that COVID-19 related coagulopathy is a distinctive entity.[20] A number of published data have wanted to quantitatively define the relationship of D-dimer levels with VTE in COVID-19. In a multicenter retrospective cohort study, Artifoni et al.[21] displayed that a D-dimer level lower than 1000 ng/ml had a 95% negative predictive value (NPV) for venous thromboembolism (VTE), which includes deep vein thrombosis (DVT) as well as pulmonary embolism (PE), while a D-dimer of greater than 3000 ng/mL had an 80% positive predictive value (PPV) for VTE. Townsend et al.[22] evaluated and noted that increased D-dimer levels (>500 ng/ml) were observed in 25.3% patients up to 4 months post-SARS-CoV-2 infection. On univariate analysis, elevated convalescent D-dimers were more common in COVID-19 patients who had required hospital admission and in patients aged more than 50 years ($p < .001$). The Global COVID-19 Thrombosis Collaborative Group designates "thromboinflammation" as an area of ongoing interest for recognizing pharmacologic targets to avert thrombosis. Increased cytokines levels consisting of IL-2, IL-6, IL-10, TNF-alpha, as well as diffuse alveolar inflammation with fibrin deposition form the basis for their investigation. It is also supposed that cytokine release may be a driver of direct as well as systemic damage associated to COVID-19. Immunomodulators targeting the complement cascade, JAK-kinase, or IL-6, with anti-thrombotic features are potential preventative choices in cases who have not yet suffered from thrombosis. To summarize, the D-dimer levels can be an important marker for predicting severity of COVID-19 based on available literature, and our findings in Indian patients also validate the same.

The study had a few limitations. The sample size of the study was small, and the study was conducted at only one study centre. Future studies can validate our study findings in Indian population, with a larger sample size and multi-centre study design. In addition, the current study was not able to assess the molecular mechanisms for the findings related with WBC, NLR and D-dimer findings.

CONCLUSION

The assessment of serial levels of WBC, NLR and D-dimer revealed significantly difference values between severe COVID and non-severe COVID subgroups. The sensitivity, specificity, and AUC for WBC, NLR and D-dimer were noted to be significant in predicting severe COVID-19. The findings indicate the utility of WBC, NLR, D-dimer in differentiating COVID based on severity, in early prognostication and categorization of patients. These novel biomarkers will therefore be further useful for screening, clinical management, and prevention of serious complications.

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