Routine blood report and common laboratory parameters in COVID-19: Experience from Bangladesh

Md. Khairul Islam1, Mohammad Murad Hossain2, Md. Mohiuddin Sharif3, Pratyay Hasan4, Md. Maruf Ahmed Molla5, Md. Robed Amin6

Abstract
Background: The present study aimed to describe the association of hematological parameters and common clinico-epidemiological features with disease severity among COVID-19 patients.

Methods: This is a hospital based observational study done in Dhaka Medical College Hospital from 01 July 2020 to 15 September 2020. Findings from hematological tests along with patient clinic-pathological features were recorded from a total of 309 COVID-19 patients. All the data were analyzed by SPSS 23.0 software.

Results: Among the studied hematological parameters hemoglobin percentage, total WBC count, lymphocyte percentage, platelet count, CRP, serum ferritin, d-dimer, and ESR were significantly associated with disease severity (p<0.05). Association was found between disease severity and other biochemical markers, such as AST, ALT, LDH, and serum bilirubin.

Conclusion: With limited resources these cheap, yet highly indicative biochemical markers could be used to assess, treat, and prognose COVID-19 patients in Bangladesh.

Keywords: COVID-19, SARS-COV-2, CBC, disease severity Bangladesh

Introduction
Towards the end of 2019, a sudden uprisingleptopneumonia cases was noted in Wuhan, China.1,2 Researchers found it to be a novel corona virus and World Health Organization (WHO) confirmed the origin by a statement issued on January 9, 2020.2,3 It was named as SARS-CoV-2 virus and the disease was named as COVID-19 by International Committee on Taxonomy of Viruses (ICTV), which was then declared by WHO on 11th January of 2020, in their 22nd situation report of the year.4,5 COVID-19 presents as an array of complicated clinical manifestations, from úu-like symptoms to multiple organ failures and death. Most common causes of mortality and morbidities are due to thrombotic complications like pulmonary embolism, DVT and stroke. Several studies have reported evidence on COVID-19 disease being associated with coagulopathy.6–8 This pulmonary coagulation and fibrinolysis due to SARS-CoV-2 infection is considered to be regulated by various pro-inflammatory cytokines and similar to pneumonia induced by other pathogens.9–11

Neutrophils are one of the most characteristic and important components of the hematopoietic, as well as immune system. It plays a very important role in inflammatory process and modified by mast cells, epithelial cells and macrophages. Neutrophil count from CBC can be uses as marker of any acute infections.12

The role of lymphocytes in both inflammation and infection is well established. In viral pneumonia the lymphocyte count is usually increased but, surprisingly, in COVID-19 characteristically its count and percentage decreases, resulting in increased neutrophil-lymphocyte, which is often associated with poor patient outcome.13 Additionally, thrombocytes play important role in the regulation of various inflammatory processes. While these parameters may be used independently as inflammatory markers, their ratios to one another may also be indicators of early inflammation.14,15

The evolving pathophysiology of COVID-19 also showed that it is a pro-thrombotic condition. Furthermore, multivariate
regression analysis in Chinese COVID-19 cohorts reported that elevated plasma levels of fibrin degradation products-dimer is associated with poor prognosis in COVID-19 patients. Parameters such as neutrophil to lymphocyte ratio, platelet count, D-dimer, and prothrombin time might also be used as useful markers for classifying and predicting the severity of COVID-19 disease. Several autopsy studies have underlined the contributions of hypercoagulability and associated inflammation in patients who died from COVID-19. The exact pathogenesis of hypercoagulability in COVID-19 is still under investigation but there are preliminary reports of endothelitis and the involvement of multimeric von Willebrand factor (VWF) with subsequent hypercoagulable status.

Another study on COVID-19 patients showed that all the parameter related to thrombosis were increased such as, factor VIII, von Willebrand factor, and fibrinogen. Other studies also have reported similar findings consistent with a hypercoagulable state, including very high d-dimer level, and increased VWF and factor VIII activity.

Bangladesh with limited resources and less developed healthcare system, often it is challenging to assess various inflammatory cytokines and high-cost genetic markers in management of COVID-19 due to exorbitant cost and limited accessibility. On the contrary, common investigations such as complete blood count (CBC) are relatively cost effective and more convenient. Hence, it is justifiable that CBC and other common laboratory investigations should hold significance for early diagnosis, management and follow-up of COVID-19 disease. Besides, few studies, up until now have systematically assessed all components of CBC and other commonly done laboratory investigations and their associations with disease severity in COVID-19 patients. Therefore, the authors hope, this study would address some of those issues and provide important clues regarding association of common biochemical parameters with COVID-19 disease.

**Methodology**

**Study design and participants selection:**

The current study is a hospital-based observational study executed prospectively in Dhaka Medical College Hospital (DMCH), Bangladesh between 01 July 2020 and 15 September 2020. The study objectivewas to collect and describe the hematological and other investigation reports from a total of 309 COVID-19 patients and look for any association with disease severity. Participants were recruited using a convenient sampling method and laboratory investigation data were collected from patient treatment sheets.

The patients were divided into four groups: mild (not included in study), moderate, severe and critical according to National Guidelines on Clinical Management of Coronavirus Disease 2019 (COVID-19).

**Operational definitions:**

- **Moderate cases**
  - Fever and respiratory symptoms with radiological findings of pneumonia
  - Respiratory distress with < 30 breaths /min
  - Pulse oxymetry showing saturation > 93% at ambient air

- **Severe cases**
  - Cases meeting any of the following criteria:
    - Respiratory distress (≥ 30 breaths/min);
    - Finger oxygen saturation ≥ 93% at rest;
    - Arterial partial pressure of oxygen (PaO2)/fraction of inspired oxygen (FiO2) ≤ 300mmHg (1mmHg = 0.133kPa)

- **Critical cases**
  - Cases meeting any of the following criteria:
    - Respiratory failure and requiring mechanical ventilation.
    - Shock.
    - With other organ failure that requires ICU care.

**Inclusion and exclusion criteria**

Individuals diagnosed as positive COVID-19 by rt-PCR method and aged >18 years were included within the study. Mild cases were excluded as at triage during admission patients with mild disease were advised to stay at home and receive symptomatic management. Patients receiving prior parenteral and or oral anticoagulant were excluded from the study. The pretested, structured case record form was used to enroll patients at admission in different wards.

**Laboratory procedures**

The blood was collected by taking all aseptic precautions. For the complete blood count (CBC), EDTA tubes and Sysmex autolyzer were used. d-dimer was assessed using tubes treated with sodium citrate, STA-R evolution coagulation analyser and original reagents (Diagnostica Stago, Saint-Denis, France). For bilirubin measurement, JM-103 transcutaneous bilirubinometer was used. C-reactive protein in serum was estimated by Nephelometric System by using commercial kit (Dade Behring BN 100, USA). Serum ferritin was measured by using XYEM enzymelinked immunosorbent assay (ELISA) test using ELISA reader. Procalcitonin measurement was performed by enzyme-linked fluorescent assay (B.R.A.H.M.S.; Diagnostica AG, Hemnigsdorf/Berlin, Germany) CBC and coagulation tests (e.g. d-dimer) were done in the Department of Hematology, and the rest of the tests in Department of Clinical Pathology, Dhaka Medical College Hospital.

**Statistical analyses**

Continuous variables were expressed as mean (± SD) and median (IQR). For comparing means, the nonparametric Kruskal-Wallis test was used. A two-tailed p-value of less than 0.05 was considered statistically significant. The authors performed statistical analysis using SPSS software (version 23.0, IBM, USA).

**Ethical approval**

No patient was interviewed during the whole study process and hence informed written consent from participants was
Results:
A total of 309 patients were included in the study, with a mean age of 51.81 (±14.44) years, and a median age of 52.00 (40.00-63.00) years. Among them, 239 (77.6%) were males and 69 (22.4%) were females. Out of these 309 patients, 178 (68.7%) presented with moderate class severity, 69 (26.6%) patients with severe class severity, and only 12 (4.6%) were identified as critical COVID-19 cases. The most common comorbidity was hypertension (63.8%), closely followed by diabetes mellitus (49.5%) and then successively by ischemic heart disease, asthma and COPD, CKD, etc. (Table-1)

Significant statistical association was observed between disease severity and hemoglobin percentage, total WBC count, lymphocyte percentage, total platelet count, ESR, CRP, ferritin, ALT, AST, d-dimer, serum bilirubin and LDH (p<0.05). (Table 2)

<table>
<thead>
<tr>
<th>Table-I</th>
<th>Baseline characteristics of the patients (n=309)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>Count (n)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
<td>69</td>
</tr>
<tr>
<td>WHO Class</td>
<td>Moderate</td>
</tr>
<tr>
<td>Severe</td>
<td>69</td>
</tr>
<tr>
<td>Critical</td>
<td>12</td>
</tr>
<tr>
<td>Hypertension</td>
<td>194</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>151</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>52</td>
</tr>
<tr>
<td>Asthma</td>
<td>38</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease</td>
<td>22</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>33</td>
</tr>
<tr>
<td>Malignancy</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table-II</th>
<th>Distribution of hematological and common laboratory parameters across the grades of severity according to National Guideline (n=309)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>Moderate (n=178)</td>
</tr>
<tr>
<td></td>
<td>Mean (±SD)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.26 (±1.78)</td>
</tr>
<tr>
<td>Total WBC (/mm3)</td>
<td>5105.91 (±5105.07)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>70.97 (±12.87)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>19.28 (±10.31)</td>
</tr>
<tr>
<td>Total platelets (/mm3)</td>
<td>922.02 (±10249.68)</td>
</tr>
<tr>
<td>ESR (mm at 1st minute)</td>
<td>33.12 (±22.89)</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>41.4 (±50.19)</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>69.81 (±507.41)</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>50.51 (±37.64)</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>36.56 (±16.12)</td>
</tr>
<tr>
<td>D dimer (μg/mL)</td>
<td>0.6 (±1.08)</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>424.65 (±221.31)</td>
</tr>
<tr>
<td>Bilirubin (μg/dL)</td>
<td>0.41 (±0.04)</td>
</tr>
<tr>
<td>Troponin I (ng/mL)</td>
<td>0.04 (±0.19)</td>
</tr>
<tr>
<td>Procalcitonin (pg/mL)</td>
<td>0.24 (±1.73)</td>
</tr>
</tbody>
</table>

*Calculated from comparing the means of the variables across 3 groups of the WHO grades of severity, employing non parametric Kruskal Wallis test. SPSS version 23.0 (IBM, USA)
Discussion
Baseline characteristics
Among the study patients, the authors found that the admitted patients are predominantly aged more than 50 years and it seems that predominantly males are the sufferer. This observation was consistently seen in previous clinical studies from China and USA. The national guideline based clinical criteria of severity was observed to be moderate in most of the cases while only 4.6 percent cases were of critical severity. This is also consistent with the prior report from China. The observation also showed that hypertension and DM was the common comorbidity in hospitalized COVID-19 cases and these were also observed in previous studies.

Common CBC parameters
Taneri et al. conducted a meta-analysis including 189 unique studies, with data from 57,563 COVID-19 patients and found pooled mean hemoglobin and ferritin levels in COVID-19 patients across all ages were 129.7 g/L (95% CI: 128.51-130.88) and 777.33 ng/mL (95% CI, 701.33-852.77), respectively. Hemoglobin levels were lower among patients with older age, higher percentage of subjects with diabetes, hypertension and overall comorbidities, and admitted to intensive care. Cavezzi et al. hypothesized that COVID-19 is more than simply a pulmonary inflammation, and there is hemoglobinopathy, dysregulation of iron metabolism causing altered oxygen transport and ultimately oxygen deprivation. Majeeed and Shajar suggested that the focus of treatment of COVID-19 would need to shift towards maintaining the oxygen carrying capacity of blood and for this purpose, blood transfusions, super-oxygenation, hyperbaric oxygen therapy could be employed. Authors in this study would like to suggest similar management among admitted COVID-19 patients.

In this study, both mean and median values for lymphocyte count decreased progressively with increasing of severity. Huang and Pranata found in a meta-analysis that patients with poor outcome have a lower lymphocyte count (mean difference “ 361.06 IL [“ 439.18, “ 282.95], p < 0.001; 12.84%) compared to those with good outcome. Zhao and Meng also reported similar findings citing a significant reduction of lymphocyte count in the severe COVID-19 group compared to the non-severe group, and those with lymphopenia have a 3-fold higher risk of developing severe COVID-19 patients.

The authors in this study also found statistical associations (p < 0.05) with total WBC count per cubic millimeter with severity of disease.

Inflammatory markers and acute phase reactants (CRP, ESR, ferritin)
In this study, mean value of CRP was different among patient groups and was found to be statistically highly significant (p < 0.001). Wang L reported that in the early stage of COVID-19, C-reactive protein levels can reflect the extent of lung lesions and disease severity and also can reflect disease changes. Chen et al. reported that the level of plasma CRP was positively correlated to the severity of COVID-19 pneumonia. Higher plasma CRP level indicated severe COVID-19 pneumonia and longer inpatients duration. Sahu et al. reported that concentrations of CRP remained high in patients who died of COVID-19 infection and could be a promising biomarker for assessing disease severity.

A pooled analysis of three studies by Lapic et al. comprising total 819 patients revealed that COVID-19 cases were associated with prominent elevations of ESR, as compared to non-severe cases. Elevation in ESR may be caused by the inflammatory process and expression of acute-phase proteins.

In this study, the mean ferritin level was 659.81 (±507.41) ng/ml and means were different among the patient groups and the association with disease severity was statistically highly significant (p < 0.001). Moreover, ferritin levels on admission were between 1.5 and 5.3 times higher in patients classified with severe disease in comparison to patients with less-severe COVID-19 disease. Vargus-Vargus and Cortés-Rojo hypothesized that by decreasing ferritin level with desferoxamine, researchers can possibly control immune dysregulation and prevent cytokine storm in COVID-19 patients. Gomez-Pastora et al. reported that average ferritin concentration was >800 ig/L for patients. They also provided similar opinion to Vargus-Vargus and Cortés-Rojo. It has been hypothesized by many researchers including, Rosário et al., Gomez-Pastora et al and Vargus-Vargus and Cortés-Rojo that ferritin may have significant role in inflammation, other than its classical role as iron storage, and it seems ferritin is related with severity of the disease. In fact, Rosário et al. described its role in other inflammatory disorders like, macrophage activation syndrome, Still’s disease, septic shock and catastrophic antiphospholipid syndrome and proposed a name for these diseases collectively as the “Hyperferrinemic Syndrome”. Here, it should be remembered that ferritin level is also commonly found high in dengue. Soundravally et al. found that the elevated ferritin level could predict the disease severity with sensitivity and specificity of 76.9 and 83.3 %, respectively, on the day of admission and the same was found to be 90 and 91.6 % around defervescence.
Liver enzymes
In this study, the mean ALT level was mildly raised and having association with severity or death. But, Zhou et al. reported that risk for in-hospital death is associated with ALT > 40 (OR: 2.87 [1.48 5.57]; P = 0.0018). Associations of ALT with severe disease in COVID-19 have been reported by many other researchers.43,51-53

In this study the authors found associations between AST and disease severity. Xu et al. showed in review of 12 articles that AST was the most frequently elevated liver function test parameter and more frequently elevated in severe diseases.54

Other laboratory findings (LDH, bilirubin, d-dimer)
In our study, the mean value of LDH was raised especially in severe cases. Henry et al. reported that elevated LDH levels were associated with a 6-fold increase in odds of developing severe disease and a 16-fold increase in odds of mortality in patients with COVID-19.55 Wu reported that LDH has good specificity to predict disease progression.56 Poggiali et al. reported that elevated LDH and CRP serum concentrations are associated to respiratory failure in COVID-19 patients.57

In this study, the mean value of d-dimer was raised and difference among the grades of severity was statistically significant (p = 0.002). Yao et al. reported that d-dimer is commonly elevated in patients with COVID-19, d-dimer levels correlate with disease severity and are a reliable prognostic marker for in-hospital mortality in patients admitted with COVID-19.58 In a meta-analysis pooling six original studies enrolling 1355 hospitalized patients Sakk et al. reported that d-Dimer levels were found to be higher in non-survivors than in-survivors.59

Study limitation:
The blood reports of sequential day of illness were not observed in this study due to lack of uniformity in all 309 cases. Besides, the Study was limited to a single hospital in Dhaka City.

Conclusion
Being acquainted with the common laboratory parameters associated with severity of COVID-19 may help clinicians & health care providers informing proper management strategies at early stage of the disease. In poor resource settings like Bangladesh, this knowledge may help diminishment of expenditure in laboratory investigations.

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Conflict of interest:
Nothing to disclose

References


