Utility of routine haematological parameters and infectious biomarkers to assess the disease severity in COVID-19 positive patients, analysis and early trend from India

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Abstract

Background and Objective- India has emerged as fifth worst hit nation by novel Corona virus pandemics in terms of total number of cases. Our literature on clinicopathological, hematopathological characteristics and trend is still miniscule. The study was conducted to assess utility of haematological parameters and infection biomarkers in identifying disease severity.

Method- The test results for complete blood counts, Coagulation screen and inflammatory markers of covid 19 positive patients were recorded and analysed based on their admission to Intensive care units, High Dependency Units or Ward. Those parameters with significant differences between the groups were assessed by receiver operating curve and the best screening cut-off was selected.

Results- 100 adults were analysed, area under the curve for total leukocyte count, Absolute neutrophil count, Neutrophil-to-Lymphocyte ratio was found >0.800, with p<0.05. The best cut off value obtained for TLC was 8100/cmm, with sensitivity and specificity of 91% and 62.5%; ANC – 6739/cmm, with 91.7% and 75% respectively; NLR - 6.9 with sensitivity and specificity 91.7% and 87.5% respectively. Haematological parameters of 33 paediatric patients studied separately and were well within normal physiological limits.

Interpretation and conclusion- study suggests that in adults, using TLC, ANC and NLR, obtained from the basic preliminary workup may segregate patients requiring intensive care at the time of admission enabling risk stratication and guide intervention.

Introduction

The corona virus disease (COVID–19) which is declared by the WHO as a pandemic in January, 2020, has now affected 216 countries, areas or territories. As of 15 Jun, 2020, more than 7 million cases are diagnosed across the globe with 0.4 million reported deaths attributed to the disease. The cases and deaths in India are on the rise, observed since March, 2020[1].

In the fight against this irrepressible jeopardy, the research priorities are hell bent on urgent identification of effective diagnostic and prognostic biomarkers of progression towards clinical worsening and mortality. Optimisation of the limited human and technical resources in the on-going pandemic is of crucial importance. Identification and use of routine laboratory parameters to the severity of the disease will contribute towards improved clinical situational awareness, to target patients at enhanced risk, optimize allocation of limited human and technical resources in the ongoing pandemic. We planned a prospective, single-centre study aimed to analyse routine haematological parameters and infection biomarkers, for assessment of severity in COVID19 positive hospital admissions. The study was conducted in a tertiary care hospital in Western India. The institution is authorized and fully equipped centre with dedicated ward, High dependency units and Intensive care units for the care of COVID–19 positive patients.
Material And Methods

Data of all patients admitted to COVID ward, ICU/HDU with confirmed positive Covid 19 status by SARS-Cov–2 Qualitative RT-PCR (Performed on Real-time cycler ABI 7500 Fast, using Pathodetect (TM) COVID 19 Qualitative RT - PCR Kit - Cat. No. PCOV100) was collected between April 16,2020 and May 25, 2020 from Laboratory Information system (LIS) of our hospital.

Data collection

The basic demographic details were collected from the admission records. Whole blood EDTA samples, sent at the time of admission were run on LH 750 / DXH 800 (Beckman Coulter) for complete blood counts. First reading of Prothrombin time (PT), activated partial thromboplastin time (aPTT), PT-Fg and D-dimer values were obtained from citrated samples run on ACL TOP–300 (Instrumentation Laboratory) sent within first five days of admission. Values for serum C-reactive protein (CRP), lactate dehydrogenase (LDH) were obtained from IMOLA (R370), Ferritin was run on ARCHITECT PLUS. To circumvent the effect of intervention if any, only the first sent investigation was included. The patients data was analysed based on their admission to Intensive care units (Severe), High Dependency Units (Moderate) or General Ward (Mild)

Statistical analysis

SPSS statistics software (version 23.0) was used for data statistics and mapping. Age was represented in median (range), and gender in frequency and percentage. The quantized variables of blood parameters were expressed as mean ± standard deviation. Levene’s test was used to assess the equality of variances between the groups. The significance between the two groups was tested by student’s t-test. $P<0.05$ was considered statistically significant in all statistical analyses. The diagnostic values of the parameters for differentiating severe cases of COVID–19 patients were assessed by receiver operating characteristic (ROC) and area under the ROC curve (AUC). Best diagnostic cut off was selected with values corresponding to maximum sensitivity and specificity.

Results

100 adults and 33 paediatric patients hospitalized to our hospital with confirmed COVID–19 RT-PCR were studied. Amongst the 100 adults there were 44 patients in ICU, 38 in HDU and 18 treated and discharged from ward. The age of these patients ranged from 25 to 85 years, with median of 51 years. Of the patients, 75 were males (75%) and 25 females (25%). The haematological parameters have been compared in table 1. The results show clear cut difference in mean values of haematological parameters between the groups. On comparing data between ward and ICU admissions, those needing ICU admissions had significantly lower Hb and ALC, while TLC, ANC, NLR, PLR were significantly higher ($p<0.05$). Platelet counts, PDW, MCV, and RDW showed no difference amongst these groups. However only
TLC, ANC and NLR were seen progressively higher in ICU admissions as compared to HDU admissions (p<0.05). P-value of all the parameters is mentioned in Table 3.

Table 2 compares coagulation profile and inflammatory markers. Coagulation screening and inflammatory markers were sent by the treating physician based on the clinical judgement and hence not available for all patients, especially so in ward patients. It was observed that mean values for LDH, CRP and procalcitonin was significantly higher (p<0.05) in ICU patients as compared to those in ward. While comparing this profile between HDU and ICU patient, rise in only Ddimer was found significant in ICU patients(p<0.05). P-value of all the parameter mentioned in Table 3.

Next, the ROC curve was used to analyze the hematological parameters with significant differences between the groups (Fig 1). The parameters with AUC>0.8 and statistical significance (P<0.05) were only considered to increase precision (table 5). Analysis showed that AUC for TLC, ANC and NLR was found >0.800, with p<0.05, and hence have an excellent diagnostic efficacy in distinguishing mild/ moderate (ward+HDU) from severe(ICU) COVID–19 cases.

The best cut off value selected for TLC is 8100/cmm, with sensitivity of 91%and specificity of 62.5%; ANC –6739/cmm, with 91.7% and 75% respectively; NLR - 6.9 with sensitivity and specificity 91.7% and 87.5% respectively.

Laboratory parameters of 33 pediatric patients were studied separately. All the patients were asymptomatic and had an uneventful clinical course during hospital stay. Only basic haematological investigation was performed along with CRP; mentioned in Table 4.

**Discussion**

The study reviewed 100 adults and 33 COVID positive children. The children were all asymptomatic and admitted to the covid ward for observation, however showed no signs of clinical or haematological deterioration. All the haematological parameters were well within normal physiological limits.

We limit our discussion to basic haematological parameters, coagulation screen and inflammatory biomarkers in Covid 19 positive adults upon hospital admission. Peak age of presentation was 55 to 60 years, with male to female ratio of 3:1. There was no significant difference in mean age and sex between the two groups (p>0.05).

Lymphocytopenia was a prominent and the most consistent feature in all affected patients, although ICU patients suffered from greater lymphocytopenia; thus significantly associated with severity. Lymphocytopenia has been conventionally known to occur in viral diseases. Affinity for the virus for lymphocytic ACE receptors may attribute to its direct cytopathic effect[2]. While lymphocyteapoptosis due to increased granulopoiesis seen as an exaggerated inflammatory response could be another reason. Studies are of the opinion that lymphopenia can be an effective predictor severity in COVID 19, our findings supports this claim[3].
Additionally rise in TLC with high absolute neutrophil count was seen significant (p<0.05) and hence a high NLR and PLR was found. High NLR may be indicative of the patient's response to inflammatory insult, with neutrophils rising in response to stress, which, when overwhelming, induces lymphocyte apoptosis [4–7]. Neutrophilshere seems act as a double edged sword. There are hypothesis that Neutrophil Extracellular Traps (NETs) released by neutrophils contribute to organ damage and death in COVID–19 patients. Also that development of Acute Respiratory Distress Syndrome (ARDS), thick mucus secretions in the airways and the development of blood clots, were similar to the symptoms of diseases already known to the researchers as being caused by NETs [8]. Fox et al, Yao et al have documented neutrophil infiltration in the pulmonary capillaries of the three autopsy samples of COVID–19 patients further supports the theory that neutrophils may be responsible for mortality in these severe coronavirus cases [9,10]. Taken together, the high ANC with associated lymphocytopenia and hence a high NLR, seems to be an affirmation of dysregulated immune system which identify patients who have lesser physiological reserve to compete the inflammatory insult. In our study, NLR was found to have the most efficient screening parameter with AUC of 0.901 with maximum sensitivity (91%) and specificity (85%) at cut off at 6.9 to identify patients likely to have severe progression. Our results are in concordance with findings of other researchers who analysed NLR as a diagnostic/prognostic/predictor of severity in Covid 19 positive patients [11].

The inflammatory markers studied (wherever possible), like CRP, Ferritin and LDH also showed a markedly increased mean value in ICU patients but did not show statistical correlation (p>0.05). Researcher from China assessed the usefulness of CRP levels in the early stage of COVID–19 and found that it positively correlated with lung lesions and could reflect disease severity [12]. Meta-analysis of reports from China have also detailed C-reactive along with D-dimers, coagulation times, and lactate dehydrogenase; with lower platelet and lymphocyte counts a common finding in "Cytokine Storm Syndrome" in hospitalized patients with COVID–19 [13]. Others have also analysed CRP to look for association with the disease aggravation showed that CRP was significantly associated with aggravation of none severe COVID–19 patients [14]. Elevated levels of these inflammatory markers found in the our study group concur to these hypothesis. However, presence of chronic comorbid conditions in many of these patients act as major confounder to establish their role in clinical worsening due to SARS-CoV 2 virus.

The mean values of Pt, aPTT, PT-Fg were not found to be significantly higher in ICU patients. D-dimer was found statistically significant in ICU group (p<0.05), when compared with HDU admissions. We refrain to compare D-dimer value of ICU patients with ward patients since most of the ward patients minimally symptomatic and were not tested.

Similar to us, results published from various studies from Wuhan, China reported elevated aPTT, elevated PT, elevated D-dimer, increased biomarkers of inflammation including interleukin–6 (Il–6), ESR, and CRP from first 99 patients hospitalized in Wuhan [15].
Report from another Wuhan hospital on the first 138 patients found minimal elevations in PT and normal aPTT, rising D-dimer in 5 non-survivors compared to 28 survivors [16]. In contrary, Tang et al studied survivor status from admission of 183 COVID–19 positive patients analyzed over 14 days, and found that over their hospitalization, non-survivors had evidence of progressive DIC with decreased fibrinogen, increased D-dimer, and increased PT, occurring 10 days after admission, although information regarding evidence of sepsis was not provided [17].

We observed no thrombocytopenia in either ICU/HDU admissions or ward patients in our study. Together with no significant rise noted in ICU admissions supports the fact that “COVID–19 associated coagulopathy” proposed to be occurring early in infection, reflects abnormalities in tests but does not fulfill the usual definition of a clinical coagulopathy where impaired ability to clot results in bleeding. The development of coagulation test abnormalities seen in SARS-CoV–2 infected patients is most likely a result of the profound inflammatory response than intrinsic procoagulant effect of virus itself. The inflammation associated with COVID–19 might subsequently activate coagulation and is the probable cause for the elevated D-dimer levels, as increased levels have been associated with many conditions other than thromboembolism, with infection an important etiology [18–20]. The acronym COVID–19 associated coagulopathy (CAC) is being used to describe the coagulation changes in infected patients, although it needs even more evidence and research to establish its exact pathology and further, its role in treatment.

Procalcitonin was also studied as a sepsis marker in these patients, though it showed a higher mean value in ICU patients but did not show statistical significance in these patients.

Considering this disease as highly contagious and pestilent, we could not correlate the coagulation profile with clinical severity of the patients. The study has a limitation that no asymptomatic carrier was included. We could not analyze and study the prognosis of the included cases. Severity of the patients was assumed on the basis of admission to ICU, and not correlated clinically.

Our study suggests that in adults, TLC, ANC and NLR can be used as a screening tool to identify patients requiring intensive care and thus enable risk stratification, guide intervention.

Declarations

Acknowledgement

We thank our department of Biochemistry for the tests of infectious biomarkers. Thanks to all health-care workers in our hospital for their efforts in caring for these patients.

Disclosure

Consent was waived off in regards to highly contagious nature of the disease. We did not include or disclosed any personal information of the patients.
Ethics Statement

The study was approved by institutional ethics committee, BVDUMC, Pune, India.

Competing Interest

The authors declare no competing interests.

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Tables

Table 1-Hematology Parameters of COVID-19 patients
Table 2 - Coagulation profile and inflammatory markers in Covid patients

| PARAMETER               | WARD (n) | Mean ± SD | HDU (n) | Mean ± SD | ICU (n) | Mean ± SD |
|-------------------------|----------|-----------|---------|-----------|---------|-----------|
| Hb, g%                  | 18       | 13.30 ± 2.21 | 38       | 11.9 ± 2.8 | 44       | 11.85 ± 2.02 |
| TLC, per cmm            | 18       | 5358.82 ± 1805.22 | 38       | 7900.0 ± 4347.28 | 44       | 10713.63 ± 496.49 |
| ANC, per cmm            | 18       | 3151.35 ± 1328.44 | 38       | 6042.1 ± 839.4 | 44       | 8907.5 ± 188.0 |
| ALC, per cmm            | 18       | 1524.64 ± 706.6 | 38       | 1158.8 ± 622.8 | 44       | 1087.8 ± 612.9 |
| NLR                     | 18       | 2.47 ± 1.60 | 38       | 6.31 ± 4.69 | 44       | 10.12 ± 6.62 |
| PLT, lakh per cmm       | 18       | 1.94 ± .43 | 38       | 2.38 ± 1.22 | 44       | 2.10 ± 1.0 |
| PLR                     | 18       | 152.05 ± 68.66 | 38       | 251.34 ± 148.25 | 44       | 246.81 ± 159.74 |
| PDW, %                  | 2        | 16.65 ± .21 | 23       | 20.49 ± 14.73 | 31       | 17.45 ± .84 |
| MCV, fL                 | 18       | 83.84 ± 12.52 | 38       | 81.53 ± 10.64 | 43       | 84.0 ± 6.8 |
| RDW-CV, %               | 18       | 15.97 ± 2.80 | 38       | 15.65 ± 2.08 | 43       | 15.45 ± 1.78 |

Total leukocyte count (TLC), Absolute Neutrophil count (ANC), Absolute Lymphocyte count (ALC), Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), Hemoglobin (Hb), Mean corpuscular volume (MCV), Red Cell volume Distribution Width-coefficient of variation (RDW-CV), Platelet count (PLT), Platelet distribution width (PDW).
| PARAMETER                | WARD (n) | Mean ± SD  | HDU (n) | Mean ± SD  | ICU (n) | Mean ± SD  |
|--------------------------|----------|------------|---------|------------|---------|------------|
| PT, seconds              | 28       | 13.20 ± 1.83 | 34      | 15.84 ± 9.22 |
| APTT, seconds            | 28       | 33.02 ± 5.73 | 33      | 35.23 ± 9.51 |
| FIB, mg/dl               | 20       | 578.95 ± 155.06 | 30      | 642.46 ± 99.53 |
| DIMER, ng/ml             | 34       | 1815.79 ± 900.58  | 34      | 7493.85 ± 14446.15 |
| LDH, IU/L                | 15       | 570.20 ± 197.39 | 30      | 1022.70 ± 1586.38  |
| FERRITIN, ng/ml          | 35       | 547.14 ± 511.77 | 37      | 701.04 ± 587.16  |
| CRP, mg/l                | 36       | 156.55 ± 197.36 | 36      | 239.48 ± 333.51  |
| PROCAL, ng/ml            | 25       | 3.72 ± 8.48 | 32      | 2.5 ± 5.6 |

Lactate Dehydrogenase (LDH), Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Fibrinogen (FIB), PROCAL (procalcitonin)

Table 3-Values of significance (p) between haematological parameters, coagulation screen and inflammatory markers
| Parameter                  | Between ICU & HDU | Between ICU and Ward |
|---------------------------|-------------------|----------------------|
|                           | Levene test (p)   | t-test (p)           | Levene test (p) | t-test (p) |
| Hb, g%                    | 0.03              | 0.92                 | 0.80            | 0.01       |
| TLC, per cmm              | 0.39              | 0.01                 | 0.004           | <0.0001    |
| ANC, per cmm              | 0.11              | 0.006                | <0.0001         | <0.0001    |
| ALC, per cmm              | 0.88              | 0.60                 | 0.46            | 0.02       |
| NLR                       | 0.08              | 0.004                | <0.0001         | <0.0001    |
| PLT, lakh per cmm         | 0.07              | 0.26                 | 0.003           | 0.37       |
| PLR                       | 0.64              | 0.89                 | 0.025           | 0.001      |
| PDW, %                    | 0.03              | 0.33                 | 0.17            | 0.19       |
| MCV, fL                   | 0.19              | 0.20                 | 0.10            | 0.94       |
| RDW-CV, %                 | 0.40              | 0.65                 | 0.01            | 0.48       |
| PT, seconds               | 0.11              | 0.14                 | -               | -          |
| aPTT, seconds             | 0.06              | 0.28                 | -               | -          |
| Fib, mg/dl                | 0.01              | 0.11                 | -               | -          |
| DIMER, ng/ml              | 0.001             | 0.03                 | 0.07            | 0.26       |
| LDH, IU/L                 | 0.30              | 0.95                 | 0.12            | 0.006      |
| FERRITIN, ng/ml           | 0.16              | 0.24                 | 2.04            | 0.59       |
| CRP, mg/L                 | 0.23              | 0.203                | 0.028           | 0.0001     |
| PROCAL, ng/ml             | 0.09              | 0.54                 | 0.35            | 0.55       |

Total leukocyte count (TLC), Absolute Neutrophil count (ANC), Absolute Lymphocyte count (ALC), Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), Hemoglobin (Hb), Mean corpuscular volume (MCV), Red Cell volume Distribution Width-coefficient of variation (RDW-CV), Platelet count (PLT), Platelet distribution width (PDW), Lactate Dehydrogenase (LDH), Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Fibrinogen (FIB), Procal (procalcitonin)

Table 4- Hematological parameters and CRP in paediatric patients
| PARAMETER               | MEAN ± SD           |
|------------------------|---------------------|
| Hb, g%                 | 11.948 ± 1.6462     |
| TLC, per cmm           | 8912.518 ± 4565.91  |
| ANC, per cmm           | 2912.12 ± 1933.38   |
| ALC, per cmm           | 5348.48 ± 3352.34   |
| PLT, lakhs per cmm     | 3.86 ± 2.55         |
| MCV, fl                | 76.41 ± 10.85       |
| RDW, %                 | 15.67 ± 2.22        |
| CRP                    | 12.40 ± 30.96       |

Table – 5 ROC analysis for significant parameters

| Test Result Variable(s) | Area  | Std. Error | p-value | 95% Confidence Interval |
|-------------------------|-------|------------|---------|-------------------------|
|                         |       |            |         | Lower Limit | Upper Limit          |
| TLC                     | 0.802 | 0.108      | 0.025   | 0.591        | 1.000                 |
| ANC                     | 0.875 | 0.090      | 0.005   | 0.699        | 1.000                 |
| NLR                     | 0.906 | 0.082      | 0.003   | 0.745        | 1.000                 |
| PLR                     | 0.604 | 0.131      | 0.440   | 0.348        | .860                   |
| D-dimer                 | 0.750 | 0.132      | 0.064   | 0.491        | 1.000                 |

Figures
Figure 1

ROC Curve to determine the area under the curve for various haematological and inflammatory parameters