Original Article

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Pathology Section

Complete Blood Count (CBC) and CBC derived Ratios in Early Diagnosis of COVID-19: A Retrospective Single-centre Study

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ABSTRACT

Introduction: Abnormalities in Complete Blood Count (CBC) are frequently observed in Coronavirus Disease-2019 (COVID-19) infection. So, CBC can serve as a simple tool for the early diagnosis of COVID-19.

Aim: To evaluate the diagnostic ability of CBC test in COVID-19 infection.

Materials and Methods: In this retrospective observational single-centred, data were collected from 102 adult non critical care patients who presented with acute fever between May 2020 and December 2020. Among 102 patients' data, 48 were found Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) positive ('cases') and 54 were RT-PCR negative ('controls'). Non parametric Mann-Whitney test was used to compare the differences in CBC. The p-value <0.05 was considered statistically significant. Receiver Operator Characteristic (ROC) analysis was used to evaluate the diagnostic accuracy of CBC tests in COVID-19. For this, RT-PCR was used as the 'gold standard' and CBC as the index test. Area Under Curve (AUC) was determined for each of the CBC tests. All statistical analysis were done using Medcalc software.

Results: The mean age of cases was 48±14 years (62% males; 38% females) and controls was 45±15 years (55% males; 45% females). Median values for haemoglobin, haematocrit, Red Blood Cell (RBC) count and Red cell Distribution Width (RDW) were significantly higher (p-value <0.05) and total White Blood Cell (WBC) count, eosinophil differential count, absolute eosinophil count, lymphocyte count, absolute lymphocyte count, immature granulocyte count were significantly lower in COVID-19 patients as compared to controls. Significant differences were observed for eosinophil (differential% and absolute) count. Almost all the platelet parameters were lower in COVID-19 patients (except Neutrophil Lymphocyte Ratio {NLR}), although the platelet count was only mildly reduced in the RT-PCR positive cases (133-475×10^3/µL; median-227.98×10^3/µL). Higher AUC values were observed with Eosinophil-differential%, Eosinophil-absolute count, Eosinophil Lymphocyte Ratio (ELR) and NLR.

Conclusion: Eosinophil count and associated ratio (Eosinophil Lymphocyte Ratio) are diagnostically useful and can serve as biomarkers for COVID-19. Further larger studies are needed to unravel the underlying mechanism and their clinical utility.

Keywords: Biomarkers, Coronavirus disease-2019, Eary recognition, Reverse transcriptase polymerase chain reaction

INTRODUCTION

Although COVID-19 was primarily documented as respiratory infection, now it is considered a systemic infection which can involve multiple systems in the body like cardiovascular, gastrointestinal, haematopoietic etc [1,2]. Various haematological changes are reported in COVID-19 patients lymphocytopenia, eosinopenia, neutrophilia and increased NLR are reported in the literature [3]. But, comprehensive data covering all CBC tests are limited. Also, recently there is an interest in utilising CBC tests in early recognition and diagnosis of COVID-19 [4].

The gold standard diagnostic test for COVID-19, RT-PCR, is fraught with limitations like technical complexity, availability of resources and turn-around-time. In order to accelerate the disease recognition especially in under-resourced settings, simpler biomarkers need to be explored. CBC or haemogram is a simple, easily available and routinely ordered haematology test.

The present study aimed at comparing the CBC test values between RT-PCR positive and RT-PCR negative patient groups and also to examine the diagnostic value of these tests for COVID-19 diagnosis as compared to RT-PCR gold standard.

MATERIALS AND METHODS

This was a retrospective observational single-centred conducted during 2021-22 on data of patients who presented to a government-designated COVID-19 facility, during the period May 2020 to December 2020. Demographic, clinical details, and laboratory details were collected from the hospital medical records department. Informed Consent and Institutional Ethics Committee (IEC) clearance (BEC-012/21) were obtained before the study was carried out.

Inclusion criteria: Adults (>18 years) presenting with acute fever and who had test results of both RT-PCR and CBC done at the time of presentation were included in the study.

Exclusion criteria: Patients requiring critical care, PaO_2 <90 and with missing clinical and laboratory data or relevant information were excluded from the study.

Sample size calculation: Sample size was calculated using the formula by Buderer N [5]. A total of 102 eligible subjects were included in the study (random sampling). A total of 48/102 were RT-PCR positive ('cases') and 54/102 were RT-PCR negative ('controls').

Study Procedure

For the present study, RT-PCR was considered as the 'gold standard test' for COVID-19 diagnosis and Complete Blood Count as the 'index test'. Following parameters were reported as part of CBC: Haemoglobin (Hb), Haematocrit (Hct), RBC count, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Red Blood Cell Distribution Width-Standard Deviation (RDW-SD), Total WBC count, Differential WBC counts, Absolute WBC counts, Immature granulocyte% and count, Atypical Lymphocyte% and count, Platelet count, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), Plateletcrit (PcT). CBC-derived ratios such as Neutrophil Lymphocyte

Ratio, Lymphocyte Monocyte Ratio, Eosinophil-lymphocyte Ratio, Platelet Large Cell Count (P-LCC) and Platelet Large Cell Ratio (P-LCR) were calculated from CBC results using Microsoft Excel.

STATISTICAL ANALYSIS

The Kolmogorov-Smirnov test was used to evaluate the variable distributions for normality. As the distribution of CBC was non normal, following statistical characteristics of CBC-lower and highest value, median and interquartile range were computed in both case and control groups. Non parametric Mann-Whitney U test was used to determine the statistical significance for differences in median CBC values between the two groups. The p-value <0.05 was considered significant. To evaluate the diagnostic accuracy of different CBC tests in COVID-19 diagnosis, ROC analysis was utilised. AUC values and sensitivity and specificity at optimum cut-offs for different CBC were determined. All the statistical analyses were performed using the statistical software Medcalc, -version 19.5.6.

RESULTS

The mean age of cases was 48±14 years (62% males; 38% females) and controls was 45±15 years (55% males; 45% females). Median values for haemoglobin, haematocrit, RBC count, and RDW were significantly higher (p-value <0.05) in RT-PCR positive cases as compared to the RT-PCR negative controls. However, no significant difference was observed for other RBC indices (MCV, MCH and MCHC) [Table/Fig-1]. With regard to WBC parameters, median values for total WBC count, eosinophil differential count, absolute eosinophil count, lymphocyte count, absolute lymphocyte count, immature granulocyte count were significantly lower in COVID-19 patients as compared to controls. On the other hand, neutrophil differential count and atypical lymphocyte count were higher in RT-PCR positive cases [Table/Fig-2]. Almost all the platelet parameters were lower in COVID-19 patients; although the platelet count was only mildly reduced in the RT-PCR positive cases (133-475×10^3/µL; median-227.98×10^3/µL) [Table/Fig-3]. Area Under Curve values >0.7

CBC test	Group	Range of values	Median (95% CI)	p-value	Area Under Curve (AUC) (95% CI)	Sensitivity (%)	Specificity (%)
Haemoglobin (gm%)	Cases (n=48)	8.2-15.8	13.3 (12.3-13.8)	0.007	0.66 (0.56-0.75)	56	72
	Controls (n=54)	7.2-15.8	12.0 (11.6-12.5)				
Haematocrit (%)	Cases (n=48)	26.2-45.6	39.3 (37.0-40.4)	0.005	0.66 (0.56-0.75)	58	70
	Controls (n=54)	20.8-45.4	35.8 (34.0-37.3)				
RBC count (X 10^{12}/L)	Cases (n=48)	3.47-5.41	4.6 (4.4-4.7)	<0.001	0.71 (0.61-0.79)	63	78
	Controls (n=54)	2.7-5.06	4.2 (4.1-4.4)				
MCV (fl)	Cases (n=48)	60.6-100.3	85.4 (84.1-87.2)	0.720	0.52 (0.42-0.62)	73	41
	Controls (n=54)	68.8-103.8	86.7 (83.0-88.3)				
MCH (pg)	Cases (n=48)	19.7-34.2	29.1 (28.1-29.7)	0.987	0.5 (0.4-0.60)	25	65
	Controls (n=54)	21.4-37.5	29.2 (27.9-29.7)				
MCHC (gm/dL)	Cases (n=48)	30.9-35	33.9 (33.6-34.1)	0.920	0.55 (0.45-0.65)	60	56
	Controls (n=54)	31.1-36.2	33.6 (33.3-3.9)				
RDW-SD (fL)	Cases (n=48)	39.4-58.7	46.8 (45.7-48.3)	0.006	0.66 (0.56-0.75)	54	76
	Controls (n=54)	41.7-57.3	44.9 (44.5-45.9)				

[Table/Fig-1]: Summary statistics of RBC tests in RT-PCR positive cases and RT-PCR negative controls.

CBC test	Study cohort	Range of values	Median (95% CI)	p-value	Area Under Curve (AUC) (95% CI)	Sensitivity (%)	Specificity (%)
Total WBC count (10^9/L)	Cases (n=48)	1.87-12.01	5.54 (5.08-6.85)	0.034	0.62 (0.52-0.72)	77	48
	Controls (n=54)	2.93-12.4	7.29 (6.34-7.81)				
Neutrophils%	Cases (n=48)	41-91.2	63.85 (59.62-67.26)	0.022	0.63 (0.53-0.73)	69	61
	Controls (n=54)	27.7-86.1	56.85 (55.8-59.63)				
Eosinophil%	Cases (n=48)	0.1-9.6	0.85 (0.47-1.45)	0.0002	0.72 (0.62-0.80)	58	85
	Controls (n=54)	0.2-15.7	2.05 (1.6-2.83)				
Basophil%	Cases (n=48)	0.2-1.4	0.6 (0.47-0.7)	0.85	0.51 (0.41-0.61)	77	30
	Controls (n=54)	0.2-1.8	0.6 (0.5-0.7)				
Lymphocytes%	Cases (n=48)	7.1-45.6	28.94 (25.22-31.85)	0.029	0.63 (0.52-0.72)	71	52
	Controls (n=54)	10.0-48.7	33.25 (29.93-34.43)				
Monocytes%	Cases (n=48)	0.4-17.5	5.35 (4.22-6.63)	0.901	0.51 (0.41-0.61)	35	78
	Controls (n=54)	0.4-12.9	5.2 (4.6-6.0)				
Absolute neutrophil count (10^9/L)	Cases (n=48)	1.37-9.34	3.54 (3.02-4.29)	0.421	0.55 (0.45-0.65)	58	56
	Controls (n=54)	1.62-9.67	3.86 (3.23-4.67)				
Absolute lymphocyte count (10^9/L)	Cases (n=48)	0.38-3.92	1.53 (1.29-1.87)	0.003	0.67 (0.57-0.76)	54	76
	Controls (n=54)	0.6-5.07	2.15 (1.80-2.55)				
Absolute eosinophil count (10^9/L)	Cases (n=48)	0.01-0.65	0.04 (0.02-0.08)	0.0001	0.72 (0.63-0.81)	75	61
	Controls (n=54)	0.01-1.18	0.16 (0.09-0.23)				
Absolute monocyte count (10^9/L)	Cases (n=48)	0.03-0.85	0.35 (0.25-0.40)	0.200	0.57 (0.47-0.67)	29	89
	Controls (n=54)	0.02-0.8	0.39 (0.34-0.42)				
Immature granulocyte %	Cases (n=48)	0-1.2	0.2 (0.1-0.3)	0.050	0.61 (0.51-0.71)	90	39
	Controls (n=54)	0-2.1	0.3 (0.2-0.5)				

Absolute immature granulocyte count (10^9/L)	Cases (n=48)	0-1.15	0.01 (0.01-0.02)	0.005	0.66 (0.56-0.75)	88	37
	Controls (n=54)	0-0.15	0.02 (0.01-0.04)				
Atypical lymphocyte %	Cases (n=48)	0-4.5	0.9 (0.77-1.15)	0.003	0.67 (0.57-0.76)	77	50
	Controls (n=54)	0-3.0	0.55 (0.4-0.8)				

[Table/Fig-2]: Summary statistics of WBC tests in RT-PCR positive cases and RT-PCR negative controls.

CBC test	Study cohort	Range of values	Median (95% CI)	p-value	Area Under Curve (AUC) (95% CI)	Sensitivity (%)	Specificity (%)
Platelet count (10^9/L)	Cases (n=48)	133-475	227.98 (205.5-258.8)	0.064	0.61 (0.51-0.70)	75	50
	Controls (n=54)	157.0-414.0	267 (244-278.3)				
Platelet Distribution Width (PDW) (%)	Cases (n=48)	8.5-16.6	10.2 (9.7-11)	0.004	0.67 (0.57-0.76)	40	93
	Controls (n=54)	8.5-17.5	11.1 (10.6-11.9)				
Mean Platelet Volume (MPV) (fL)	Cases (n=48)	7.1-10.4	8.8 (8.3-8.9)	0.005	0.66 (0.56-0.75)	75	57
	Controls (n=54)	7.3-10.8	9.2 (8.8-9.5)				
Plateletcrit (%)	Cases (n=48)	0.13-0.41	0.19 (0.18-0.22)	0.010	0.65 (0.55-0.74)	77	57
	Controls (n=54)	0.15-0.37	0.24 (0.22-0.25)				
Neutrophil Lymphocyte Ratio (NLR)	Cases (n=48)	0.9-12.8	2.2 (1.9-2.7)	0.031	0.62 (0.52-0.72)	35	87
	Controls (n=54)	0.6-8.4	1.8 (1.6-2.1)				
Platelet-Large Cell Ratio (P-LCR)	Cases (n=48)	14.9-39.9	26.7 (24.4-28.4)	0.005	0.66 (0.56-0.75)	73	56
	Controls (n=54)	16.4-45.0	30.3 (28.3-33.7)				
Platelet-Large Cell Count (P-LCC) (10^9/L)	Cases (n=48)	37.0-135.0	58 (54.0-63.5)	0.001	0.69 (0.60-0.78)	65	74
	Controls (n=54)	41.0-130.0	75.0 (68.0-79.7)				
Lymphocyte Monocyte Ratio (LMR)	Cases (n=48)	1.7-26.7	5.1 (4.3-6.2)	0.351	0.55 (0.45-0.65)	60	59
	Controls (n=54)	2.2-69.8	6.1 (4.9-7.1)				
Eosinophil Lymphocyte Ratio (ELR)	Cases (n=48)	0.01-0.3	0.03 (0.02-0.05)	0.001	0.71 (0.61-0.80)	52	85
	Controls (n=54)	0.01-0.7	0.7 (0.05)				

[Table/Fig-3]: Summary statistics of platelet tests and CBC-derived ratios in RT-PCR positive cases and RT-PCR negative controls.

were observed for Eosinophil% (0.72), Absolute Eosinophil Count (0.72), Eosinophil Lymphocyte Ratio (0.71) and RBC Count (0.71).

DISCUSSION

Early diagnosis of COVID-19 is essential on account of its high infectivity and mortality. Though RT-PCR is the gold standard for diagnosis, it is limited by technical complexity and delays in turn-around time. The current study examined the CBC and its derived ratios for early recognition of COVID-19 as they are simple, inexpensive, easily available and routinely ordered test. Significantly higher values for haemoglobin, haematocrit, RBC count and RDW were observed in RT-PCR positive cases. The present study was similar to study by Guan WJ et al., Liu X et al., Xu XW et al., and Usul E et al., [6-9]. However, Yuan X et al., and Mei X et al., reported lower values in critically ill and severe COVID-19 patients [10,11]. This difference is attributable to the severity of the disease and other associated co-morbid conditions. The study cohort included only mild COVID-19. There was no difference in MCV, MCH and MCHC between the two groups. As many authors didn't report on these indices, authors could not compare present study findings with others [12-15].

There was a significantly lower total WBC count, lymphocyte count (differential and absolute) and eosinophil count (differential and absolute) in RT-PCR positive patients. There was a significant increase in neutrophil differential count in RT-PCR positive group, but there was no difference in the absolute neutrophil count between the groups. This was in agreement with few other studies [16-18]. There was an increased atypical lymphocyte count (both differential and absolute) and decreased immature granulocyte count (both differential and absolute) in the case group. Though few studies have described abnormalities in WBC morphology in peripheral smear, there were no published studies on these analyser-derived parameters to compare with [16,17]. Among the CBC parameters, changes in eosinophil count were the most significant. In fact, the eosinophil differential% and absolute eosinophil count had the highest AUC values among all the CBCs, indicating that they are the most important discriminatory tool in the early recognition of COVID-19. This was similar to a study by Soni M, where eosinopenia was found to be a diagnostic and prognostic marker with as much as 78% of patients having low or zero eosinophil count [16]. Platelet count was significantly lower in RT-PCR positive patients; so, also the other platelet indices-MPV, PDW and PcT. Similar findings were observed by Ozcelik N et al., Rahman A et al., observed that though thrombocytopenia was found in 5-21% of COVID-19 patients, the severity was less compared to other viral infections like Dengue [19,20]. With respect to CBC-derived ratios, NLR and ELR had the highest AUC for COVID-19 diagnosis. This was comparable to a study by Yang H et al., on CBC parameters (lymphocyte count, neutrophil count, monocyte count, NLR and LMR) who observed highest AUC for NLR and also found it to be prognostically useful. However the study did not include eosinophil or its derived ratios [21].

The AUC values were the highest for absolute eosinophil count, eosinophil%, eosinophil lymphocyte ratio and RBC count making them potential markers in recognising COVID-19. The commonly tested NLR had an AUC of 0.62 in the present study, but had the highest specificity among the CBC tests and CBC-derived ratios.

Limitation(s)

The present study was a retrospective study and did not attempt to explore the dynamic changes of the test values or their prognostic utility.

CONCLUSION(S)

Significant haematological changes occur in COVID-19 patients. Lymphocytopenia, neutrophilia and eosinopenia are observed in COVID-19 individuals. Eosinophil Count (differential% and absolute count) and CBC-derived ratio ELR are the most promising markers useful in the early recognition of COVD-19 in addition to NLR.

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