A Study of Hematological Parameters along with CD 4, CD 8, CD 19 and CD16+CD 56 Lymphocyte Counts in COVID-19 Positive Patients

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Abstract: *Coronavirus disease (COVID-19) is a novel disease with world wide spread and varied clinical presentation. Studies are required to understand the pathogenesis of the virus and diagnostic parameters useful in diagnosing and predicting the prognosis of the disease. **Aims & Objectives:** Our Aim was to evaluate various hematological parameters in patients with mild and moderate/severe disease. **Materials & Methods:** COVID-19 positive patients are grouped into mild and moderate/severe categories based on the symptoms. Their blood samples were analysed for TWBC, ANC, ALC, RNC, RLC, PLT, CD4, CD 8, CD19 and CD56 cell counts and statistically analysed for P value. **Results:** A total of 100 patients, 50 mild and 50 with severe symptoms were included in the study. Severe cases had lymphocytopenia with reduced CD4 and CD56 counts. TWBC, ANC, ALC, RNC, RLC, CD4, and CD16+CD56counts showed significant P value. whereas PLT,CD8 and CD19 were not significant. **Conclusion:** COVID-19 impairs immune system by reducing the lymphocyte counts. This effect is more in severe cases causing tissue damage. Diagnosing the disease at an early stage would help in early intervention and better recovery of the patients. Hence the above hematological parameters can be prognostic indicators of COVID-19 positive patients.*

Keywords: COVID-19, Lymphocyte counts, Total WBC count

1. Background

The outbreak of novel corona virus is explained as one of the most severe viral infections mankind has seen because of its spread, morbidity and mortality it is causing. Corona virus disease (COVID-19) is caused by corona virus2 (SARS-Cov-2) (1). Several studies are being conducted worldwide to understand the pathogenesis of the disease, clinical presentation and thereby the cause for mortality in the affected patients. Scientists of various eminent research groups have come up with several hypothesis but most of them need extensive population based studies to be proved. Understanding the disease process is the need of the hour as this forms the base for therapeutic management. Though it is the same virus everywhere, the statistical figures regarding the incidence and the rate of mortality are showing vast geographical variations. Hence studies should be done in every country, independently, to understand the behavior of the disease and develop statistical data pertaining to their subset of population. Diagnostic parameters provide an insight into the disease activity in the diseased individuals (2). In the present study we have evaluated various relevant hematological parameters like Total WBC count (TWBC), Absolute Lymphocyte count(ALC), Absolute Neutrophilic count (ANC), Platelet count(PLT) and Relative neutrophil percentage (RNC), Relative lymphocyte percentage (RLC) along with CD4, CD8, CD19 and CD16+56 lymphocyte counts in confirmed COVID-19 positive patients with mild and moderate/severe disease. The values were statistically analysed.

2. Materials and Methods

The present study is a cross sectional study conducted for a period of 3 months. Institutional ethical committee clearance was acquired before starting the sample collection. The sample size was 100 which included COVID-19 RT-PCR positive patients. All the patients were adults who had no other co-morbidities other than hypertension. Pregnant women, children and patients with co-morbid conditions were excluded from the study. Patients were categorized into two groups –

Group 1: Mild (Non-ICU) and Group 2: Moderate/Severe (ICU) cases depending on the symptoms at the time of admission as per the regulations formulated by ICMR, 2020. Mild cases are patients with Fever, cough, malaise, rhinorrhoea, sore throat without shortness of breath. Moderate/Severe cases are patients with Respiratory rate >24/min, SpO2 <94% in room air and require ICU admission. Informed consent was taken from the patients. EDTA samples of these patients were run in 5 part coulter (SYSMEX XN 1000) and TWBC, ANC, ALC, RNC, RLC and PLT were recorded. The sample was also run in flow cytometer for CD4, CD8, CD19 and CD16+CD56 cells. The results were then compared and statistically analysed by SPSS soft ware version 22. We did independent samples t test to derive a P value. P value <0. 5 was considered significant.

3. Results

A total of 100 COVID-19 positive patients were included in the study. Group 1 comprised of 50 patients who had mild symptoms and Group 2 comprised of other 50 cases that had moderate to severe symptoms and required ICU admission. The mean age of the patients in Group 1 was 44.56 years where as the mean age in Group 2 was 50.6 years. The mean age group was high in patients who required ICU admission. The hematological parameters in 5 part cell counter and CD cell counts in flowcytometry were compiled and statistically analysed. Number of patients with normal, decreased and

increased values for each parameter were tabulated as follows:

Table 1: Results in Group 1(Mild cases)

MILD (50)	Normal	Decreased	Increased
TWBC	38	4	8
RNC	43	4	3
ANC	48	0	2
RLC	38	11	1
ALC	44	5	1
CD4	40	10	0
CD8	24	25	1
CD19	37	1	12
CD16CD56	11	39	0
PLT	40	1	9

Table 2: Results in Group 2(Moderate/ Severe)

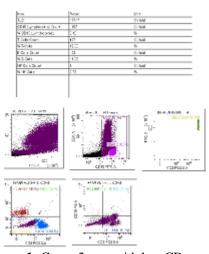| SEVERE(50)	Normal	Decreased	Increased
TWBC	37	1	12
RNC	29	4	17
ANC	35	1	14
RLC	22	28	0
ALC	32	18	0
CD4	27	22	1
CD8	14	31	5
CD19	38	4	8
CD16CD56	8	42	0
PLT	34	2	14

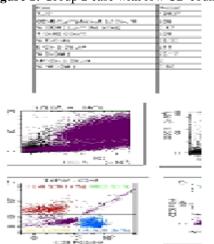
Figure 1: Group 2 case with low CD counts

Figure 2: Group 2 case with low CD counts

There was no major difference in TWBC count in between the two groups. Majority of the cases had counts within normal limits in both the groups though few ICU patients showed higher counts. TNC was normal in majority of the Group 1 patients but a significant number of ICU patients (34%) showed raised TNC. Similarly, ANC was also high in good number of Group 2 patients (28%). TLC was decreased in 56% of the cases in Group 2 where as in Group 1 cases only 22% had decreased TLC. Similarly ALC was decreased in significant number (36%) of Group 2 patients. Whereas, majority (88%) of Group 1 patients had normal ALC. There was absolute lymphopenia in patients with severe clinical symptoms. CD4 cell count was decreased in almost half (44%)of the Group 2 patients but only 22% of Group 1 cases showed a fall in CD4 counts. CD 8 counts decreased in 50% of the patients in Group 1 category as well as 62% of Group 2 cases. On the contrary, CD 19 cells increased in 24% of Group 1 cases and in 18% of Group 2 cases. CD16+CD56 cell counts were decreased in majority of the patients in both categories, 79% in Group 1 and 84% in Group 2 category respectively (Figure 1 &2). None of the patients showed increased CD56 cell counts. Platelet counts were mostly normal in both the groups. Only one patient inGroup1 and 2 patients in Group 2 showed thrombocytopenia.

Table 3: Statistical comparison of parameters in Group 1 and Group 2

	group	N	Mean	Std. Deviation	Std. Error Mean	Sig. P Value
TWBC	1	50	7. 3294	2. 48834	0. 3519	
	2	50	9. 1178	5. 72853	0. 81836	
ANC	1	50	4. 8424	2. 08656	0. 29508	. 003
	2	50	7. 3294	5. 29175	0. 75596	
ALC	1	50	1. 8114	0. 68389	0. 09672	. 042
	2	50	1. 501	0. 81376	0. 11625	
RLC	1	50	26. 074	8. 87424	1. 25501	. 000
	2	50	17. 5578	9. 51228	1. 3589	
PLT	1	50	345. 22	148. 04898	20. 93729	. 747
	2	50	335. 7347	143. 14835	20. 44976	
RNC	1	50	63. 616	11. 43361	1. 61696	. 000
	2	50	73. 6429	12. 32149	1. 76021	
CD4	1	50	701. 78	307. 9142	43. 54564	. 010
	2	50	529. 3265	344. 25853	49. 17979	
CD8	1	50	367. 9	180. 94009	25. 58879	. 184
	2	50	314. 4286	215. 48637	30. 78377	
CD19	1	50	340. 6	235. 26693	33. 27177	. 252
	2	50	291. 0612	189. 48245	27. 06892	
CD56	1	50	77. 4	46. 47273	6. 57224	. 008
	2	50	51. 8776	47. 97683	6. 85383	

The mean of TWBC in Group 1 was 7.32cells/cumm. The highest count was 12.56 in this group. The mean TWBC in Group 2 was 9.11 cells/cumm. The highest count was 23.48 cells/cumm with ANC of 21.45. The mean ANC of Group 1 was 4. 84 where as that of Group 2 was 7.32, higher than the mild category. The mean RNC in Group 1 was 63. 6% where as it was 73.6% in Group 2, highest being 91%. The RLC in Group 1 was 26% and 17.55% in Group 2. ALC count in Group 1 was 1.81cells/cumm where as that of Group 2 was 1.50 cells/cumm. Thus, Absolute lymphocyte counts did not show much difference in between the two groups. The mean count of CD4 in Group 1 was 701/ul and it was less Group 2, 530/ul. The least CD4 cell count was 79 cells and the same patient also had decreased CD8, CD19

and CD16+CD56 cells along with absolute lymphocytopenia. The mean CD8 count in Group 1 was 368/ul and that of Group 2 was 314/ul. There was not much difference between the two groups. The mean CD 19 counts in mild category was 340/ul and it as 291/ul in Group 2. Mean CD16+CD56 count in Group 1 was 77.4/ul and in Group 2 was 51. 8/ul. The mean platelet count in Group 1 was 345/ul and Group 2 was 335/ul. There was not much difference between the mean platelet count between the two groups. P value was significant (<0. 5) for TWBC, ANC, ALC, TNC, TLC, CD4, CD56. where as P value was not significant for PLT count, CD8 and CD 19.

4. Discussion

COVID-19 positive patients have varied symptoms (5) and their management depends on presentation at the time of admission. In addition to symptoms, certain laboratory parameters can also be indicators for the clinical behavior of these patients (5). In our study, there wasn't much difference in the TWBC amongst the two groups. However leucopenia was observed in very few patients. Bingwen et al, in their study, observed leucopenia in 29. 2% of the ICU patients (2). Similar were the findings in a study done by Rajab mardani et. al. (6). ANC was high in significant number of cases in Group 2. According to Bingwen et. al. ICU patients tend to have neutrophilia which could be due to hospitalization (2). Lymphocytes have a pivotal role in viral illness as the whole pathogenesis is immunity related. In our study, a decrease in ALC was found in Group 2 patients compared to Group 1 patients. Lymphocytopenia was a common finding in most of the studies on COVID-19 positive cases (6, 7). Lymphocytopenia can be a feature of any viral infection. Hence we further evaluated the subsets of Lymphocytes. CD 4 counts were normal in most of the mild cases where as ICU admitted patients showed a fall in these counts in many of the patients. Similar findings were seen in study done by Yishan Zheng et. al. (3). CD4 lymphocytes are important for cellular and humoral immunity and hence a variation in their counts is expected in COVID-19 positive patients (8). In our study, a decrease in CD 4 counts in ICU patients suggests that they may be related to the increased severity of the disease. CD 8 cell counts were also decreased in ICU patients but statistically it was found to be not significant. Similarly CD 19 cells were normal in most of the cases in both the groups and the readings were statistically insignificant. This finding may be significant while considering plasma therapy for COVID-19 positive patients. CD 19 positive cells are responsible for antibody production in the human body. If these counts are normal in these patients then the antibody production should not be hampered. Hence we can hypothesise that plasma therapy in patients with normal CD 19 count patients can be only an adjuvant but not therapeutic. However, such studies have to be done on a larger sample size by assessing the antibody titers before and after plasma therapy (9). CD16+CD56 cells decreased in both the groups but the percentage of cases was little more in ICU patients. CD56 cells, the natural killer cells are an important component of the immune system to combat infections. Several studies have been done in various diseases to define their functions and their role in fighting the viral infections (9). A decrease in these cells would derail the immunity of the individual and provide an environment which favours the viral cytopathic effects. Platelet counts showed no major difference in both the groups and statistically also it was insignificant. Several studies have shown that, it is the lymphocytes and their subsets which show variations in COVID-19 positive cases (10). Being a viral infection, as the severity of the disease increases these cell counts decrease (11, 12) and make the body immune system less efficient. Ruyum He, Zilong et al, in their study, have stated that the use of glucocorticosteroids in these patients should be in relation to the lymphocyte counts (14). Hence, the evaluation of these parameters at the time of admission would help in predicting the behavior of the disease and plan treatment protocols.

5. Conclusion

In COVID-19 positive patients no single parameter is efficient enough to predict the disease course, but a combination of these parameters would definitely be a valuable prognostic indicators. We also recommend similar studies with larger sample size to be done in various population groups to substantiate our inferences. This will also benefit the clinical trials pertaining to use of immunomodulators as a part of therapeutic regimen in COVID-19 positive patients.

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