Egyptian Journal of Medical Microbiology Volume 30 / No.1 / January 2021 93-99 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Correlating COVID-19 Infection Severity and Prognosis with Cytokines Level

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Key words:
Coronavirus 2019, SARS-CoV2, COVID-19, lymphopenia, eosinopenia, cytokine storm, immunoparemeteres

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ABSTRACT

Background: *It is noted that severe acute respiratory syndrome caused by coronavirus (SARS-CoV2) is considered one of the most important issue under research nowadays. Most patients reveal mild flu like symptoms while some are asymptomatic, there is a considerable percentage that requires special hospital care that may extend to intensive care. Upon studying immune system response to COVID, both innate and adaptive immunity had role in defense against infection, involving leucopenia with all types of T cell low count associated with abnormal rise of the cytokine level the what so called cytokine storm which lead to severe lung damage and severe respiratory symptoms.* ***Objectives:*** *Our study aims to classify and identify prognosis of disease by examining selected cytokines and correlating them with severity of symptoms that could help to predict course of disease in patients and detect clinically their improvement. Also by analyzing different immune parameters, novel therapeutic ways could be detected.* ***Methodology:*** *COVID-19 Confirmation was based on RT-PCR along with blood analysis involving differential measurement of leucocytic count and ELISA measurement of selected cytokines was done.* ***Results:*** *Data obtained in our study from SARS-CoV-2 infected patients have shown that most cases had been characterized by a cytokine storm with elevated levels of IL-1, IL-6 and TNF-α respectively that decline upon improvement of clinical conditions.* ***Conclusion:*** *The novel coronavirus mainly acts on lymphocytes, especially T cells. T cell types analysis especially easinophils and lymphocytes can help in early diagnosis of cases. Cytokines level monitoring is of great benefit in early screening, diagnosis, and classification of patients before clinical symptoms and complications arouse where prognostic features will be available for future patients outcomes whether complete cure or ICU admission will be required based on his cytokine profiling system.*

INTRODUCTION

The novel 2019 coronavirus (SARS-CoV2) had been classified as a worldwide pandemic with increasing infections associated with rise of mortality rates.

It is well known that immune system plays a major role in defense and pathogenesis of disease [1].

By analysis of immune system output we can understand prognostic markers that could predict possible disease outcome and provide early detection and classification for patients, also can provide possible treatment for the disease and its complications [2,3].

Upon studying COVID-19 immunological response two phases were detected:

Early incubation and mild stage where a specific cellular immune response acts to decrease viral proliferation and cause virus elimination.

And a late stage where severe cases occurs with high morbidity rates where the cytokines take the upper hand and cause cytokine storm that promotes lung damage [1].

Therefore immune system is a double edged weapon where it is essential to clear the virus whereas it causes severe pathological changes to the host [4].

Upon binding of COVID-19 to TLRs (TLR-3, 4, and 7) activation of release of IL-1β and IL-6 occurs. Both cytokines are involved in arousal of well-known symptoms of malaise, fever, myalgia also leading to lungs inflammatory conditions [5].

Also, C-reactive protein (CRP) elevation with other cytokines rise, IL-2R, IL-6, IL-8, IL-10, and TNF-α, were found in advanced cases [6,7].

Some literature suggested cytokines inhibitors as a novel therapy for severe cases to abort cytokine storm and prevent further lung damage [6,8].

METHODOLOGY

Our study was conducted in Cairo University Hospitals during the period of March to June 2020. The study was conducted in accordance with the ethical

DOI : https://doi.org/10.51429/EJMM30112

principles of the Helsinki Declaration and ethical approval was given by local Ethics Committee.

Oral or written consents were obtained from 350 patients with COVID-19 patients where the clinical and immunological characteristics were analyzed.

In our study we aimed to collect major immune markers changes during disease course with correlating their level to clinical picture of patients.

- Besides clinical evaluation, the level of, IL-1B, IL-6 and TNF alpha were measured.
- Blood samples were analyzed involving differential measurement of leucocytic count.
- CIOVID-19 confirmation was based on RT-PCR also chest CT was done and graded by CO-Rads system.
- Treatment decisions for all patients were taken usually within the first week after the admission where the laboratory tests were repeated.
- Follow up blood tests were repeated on day 2, 14-40 compared with clinical improvement or deterioration.

Immunocompromised and patients with chronic diseases were excluded from our study.

When the laboratory parameters were available, the patients were classified into two groups: the first group comprised 150 cases who developed a serious COVID-19 disease.

A second group of 200 cases who show mild symptoms who were discharged with follow up visits to asses their clinical manifestations and record their repeated laboratory tests

Statistical Analysis:

Variables were reported as mean and standard deviation or median and interquartile range (IQR), as appropriate, or frequency rates and percentages if categorical; consequently, comparisons between 2 groups were made by parametric tests (t-test for two independent samples) or no parametric tests (Mann--Whitney test) for continuous variables.

Proportions were compared by $\chi 2$ test, or Fisher exact test. Bivariate correlation was made by two tailed Pearson or Spearman tests.

All statistical analyses were performed using SPSS version 15.0 software (SPSS Inc.). For unadjusted comparisons, a 2-sided α of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

It had been recorded that COVID-19 clinical manifestations usually arouse after 6-8 days with commonly seen symptoms like fever, coughing, sore throat, fatigue, possibly diarrhea, or dyspnea [9].

As for radiological findings, Computed tomography chest images for COVID-19 patients with severe complications reveal the presence of pulmonary ground-glass opacities of pneumonia that may be seen in sub pleural regions in both lungs or in single side [10].

Laboratory findings reveals lymphopenia is a main feature of patients with COVID-19, especially in severe cases where lymphocyte percentages were found to be lower than 20% in severe cases 4.

Further analysis showed a significant decrease in T cell counts, especially $CD8^+$ T cells in severe cases compared with mild cases [11].

Qin et al.[12] reported that the percentage of memory helper T cells ($CD3^+CD4^+CD45RO^+$) is also decreased in severe cases compared with non-severe cases.

In our study lymphopenic patients constitute 60 % of all studied patients where severe cases show more decline in the absolute number of lymphocytes with a count of 147 patient which is approved by most of other studies while 66 patients of mild cases group show decline in count, similar results were reported in many trials [1,12,13,14,15]. (Table 1 & Fig. 1)

Table 1: Follow up lymphopenic count and easinophilic count in severe and mild COVID cases groups

	severe cases 150	**mild cases 200**	**Total studied patients (350)**
easinophil count day 2	100(67%)	60(33%)	160 (45%)
easinopenia day 14-40	56 (37%)	20 (10%)	84(24%)
lymphopenia day 2	147 (98%)	66 (33%)	217 (60%)
lymphopenia day 14-40	27(22%)	3 (4.5%)	30 (8%)

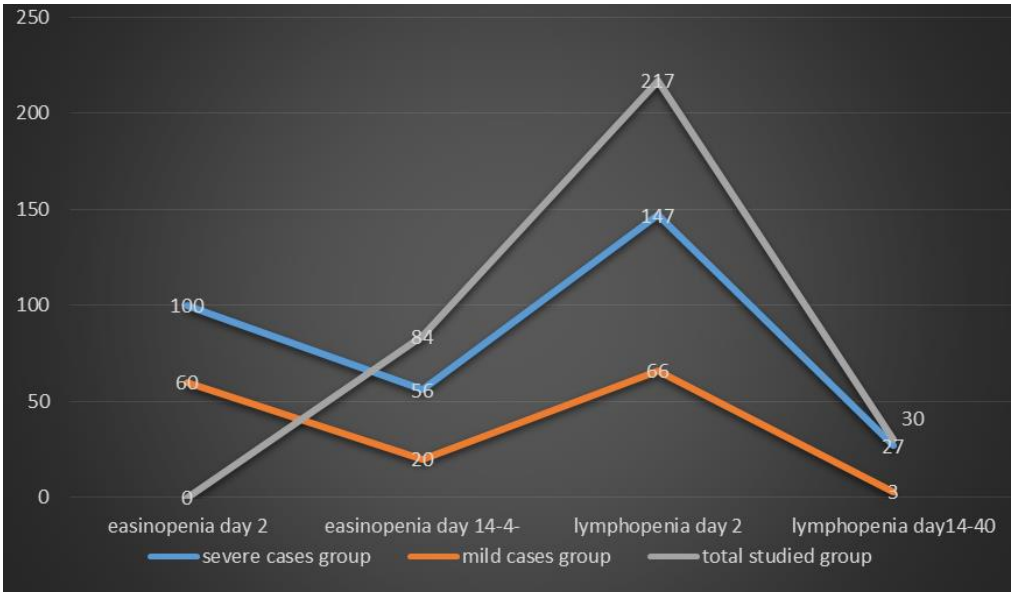

Fig. 1: Follow up lymphopenic count and easinophilic count in severe and mild COVID cases groups

Guan et al.[16] reported that (83.2%) of patients had lymphopenia.

Upon correlating lung pathology by imaging it was noted that severe lymphopenic patients had more pathological lung changes than patients with mild lymphopenia, this finding was approved by another study[17].

In our study lymphopenia was markedly recorded in older age than young adults which was approved by other studies [18,19].

A possible explanation is that young age can produce broad reactive antibodies, presence of physiologic lymphocytosis and differences in lymphocyte compartment, virus-to-virus interactions in the airways (simultaneous presence of other respiratory viruses in the airway mucosa), differences in the expression of ACE2 [2,4].

These data indicate that lymphopenia can be used as an indicator of disease severity and prognosis of patients with COVID-19. Nevertheless, lymphopenia was present in some non-severe and pregnant cases[2,6,8,9,11].

Both cases group show rise in lymphocytic count upon recovery from respiratory symptoms to reach 78% and 95.5% in severe and mild cases respectively (fig 1).

Another finding is the eosinopenia associated with COVID-19 infections reported by several author groups.

Recent findings suggest that eosinophils have important antiviral properties[19,20] as they had an anti single-stranded RNA viral properties, also eosinophils are able to produce nitric oxide and can induce CD8+ T cell proliferation and activation as a response to virus- or viral-peptide exposure [20].

Taking into account all these facts, the reported eosinopenia in COVID-19 patients is of special interest.

Upon measuring easinophils along our study marked easinopenia was found in 100 cases of severe cases groups while 60 patients of mild group show easinopenia (table1) increase in eosinophils count was observed during treatment in 44 cases and 40 cases of severe and mild cases groups respectively (fig. 1), which was consistent with other studies[21].

Li et al.[22] compared 10 COVID-19 patients with 30 patients affected by other viral pneumonia. They found that leukopenia, lymphocytopenia, and eosinopenia were more common in COVID-19 patients compared to non-COVID-19 subjects [22].

Another paper report [23] stated that eosinopenia with lymphopenia may be a potential indicator for COVID-19 with both diagnostic and prognostic value in a study carried on 140 hospitalized patients.

On the other hand, consistent decline in eosinophils show unfavorable progression of COVID-19 and severe lung pathology as shown in their chest imaging, this finding had been approved by other studies who recorded rapid lung deteriorating function with declining easinophilic count [24].

Combination of eosinopenia together with elevated high-sensitivity CRP could effectively triage suspected patients with COVID-19 from the other patients with fever[22].

It seems that eosinophils role in COVID-19 has more diagnostic and eventually prognostic value than real participation on the COVID-19 pathology [24,26].

As for cytokines level, it is recorded that IL-1 plays a role in inflammatory response to infection being released from macrophages [25].

SARS-CoV-2 was found to act on maturation of IL-1β, with subsequent activation of other proinflammatory cytokines, such as IL-6 and TNF-α. [25-27].

In our study IL-1 was measured along course of disease starting from symptoms arousal until complete cure with comparing its level with clinical improvement and chest imaging scale system.

Upon measuring IL-1 in our cases it showed elevated levels in both mild and severe cases groups with massive rise in severe lung pathology cases with widespread area of lung opacities where 87 % of severe case patients in our study showed uprise of IL-1B while only 22 % of mild group show Il-1B rise (table2 and fig 2). This agreed with most of others as Yang et al. 26 who stated elevated levels of the antagonistic receptor of IL-1 (IL-1Ra) in 14 severe cases of COVID-19, which were associated with increased viral load, progressive lung damage, and mortality rate.

Table 2: Follow up cytokine level count in severe and mild COVID cases groups.

	Severe cases 150	**Mild cases 200**	**Total studied patients (350)**
IL-1 day 2	130 (87%)	44 (22%)	174 (49%)
IL-1 day 14-40	30 (20%)	7 (3.5%)	37 (10.5%)
IL-6 day 2	138 (92%)	70 (35%)	208 (59%)
IL-6 day 14-40	13 (8.6%)	5 (2.5%)	18(5%)
TNF α day 2	115 (76%)	108 (54%)	223 (63%)
TNF α day 14-40	17 (11%)	9 (4.5%)	26(7.4%)

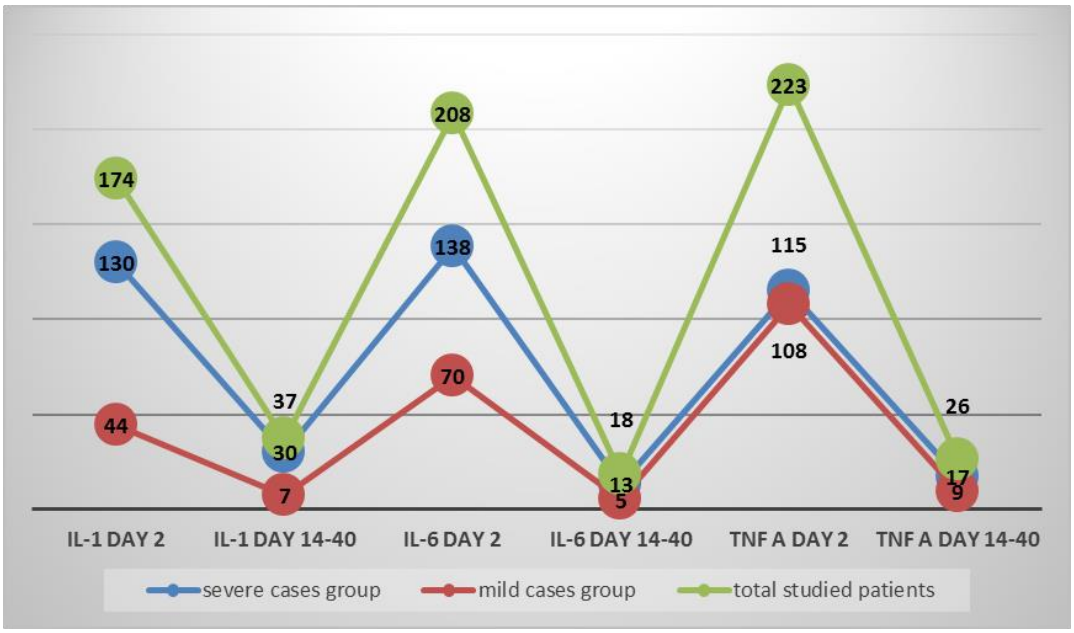

Fig. 2: Follow up cytokine level count in severe and mild COVID cases groups.

This was consistent with the study of Wen et al.[17] who reported an increase of CD14+ monocytes with inflammatory gene expression as well as abundance of CD14++IL-1β+ [17].

Liu et al.[20] also found elevated IL-1α levels in patients with severe COVID-19, and these were strongly associated with lung injury.

Upon viral clearance detected by PCR in our study declination of IL-1 level was detected to reach near normal levels in both patients groups with left over high level in 30 severe case group and only 7 in mild cases group (table 2).

As for IL-6 it is well known to be secreted by multiple immune cells which plays a role in generating acute-phase proteins, including C-reactive protein (CRP), serum amyloid A (SAA).

Upon measuring IL-6 level in our study elevated IL-6 levels have been observed in positive cases and closely related to the severity of symptoms (fig 2).

In both groups elevated IL-6 levels have been found in patients with COVID-19 with very high levels being recorded in 92% of severe cases groups that progressed to severe lung damage in 20% of that group while 35% of mild cases groups showed IL-6 rise.

This was consistent with Yang et al.[26] who detected elevated IL-6 levels in one-third of patients with mild symptoms and three-quarters of those with severe symptoms, concluding that IL-6 may be of prognostic value in patients with COVID-19.

Also Among patients admitted to ICU, Diao et al.[27] found an inverse proportional association between elevated IL-6 levels and T cell counts.

IL-6 levels were also found to be markedly higher in patients who died from COVID-19 than in those who recovered [28].

As for TNF-α it has been closely related to proinflammatory responses mediated by IL-1β and IL-6, also in the regulation of inflammatory processes and infectious diseases [29].

We had observed that serum TNF-α levels are elevated in all patients with COVID-19 and was recorded to reach a higher level in 76% of severe cases group where overproduction was related to a poor prognosis in COVID-19 patients progressing to massive lung damage while 54% of mild cases group show its rise.

Diao et al. [29] reported similar results in a sample of 522 patients with COVID-19 and found an inverse relationship between TNF-α levels and T-cell counts.

In contrast, another report described normal TNF-α levels in patients with COVID-and MERS[30].

Data obtained in our study from SARS-CoV-2 infected patients have shown that most of severe cases had been characterized by a cytokine storm whereas 87%, 92% and 76% show elevated levels of IL-1β. IL-6 and TNF-α respectively.

In comparison to mild group cases where 22%, 35%, and 54% show elevated levels of IL-1β. IL-6 and TNF-α respectively.

The further analysis has shown a decline in the above mentioned levels until reaching normal level with clinical improvement in severe cases with percentage 80%, 91.4% and 89% in IL-1β. IL-6 and TNF-α parameters respectively, while drop to normal levels in almost all cases of mild group cases (fig 2).

Correlating cytokine profile with chest radiological finding was done in our study, and it is recommended for further study, showed that the lungs typically demonstrate ground-glass appearance with variable locations, Broncho vascular thickening and septal thickening with consolidation in severe cases group.

Radiological image was related with IL-1β, IL-6 and TNF- α upon admission and in follow up after few days. CT findings evolve over time with normal CT scans during the first 3–4 days progressing to septal thickening and increased ground glass opacities appear with rise of measured cytokines deteriorating within 2 weeks period to show full blown pictures of consolidation and vascular thickening in severe cases group as agreed by other studies [31,32,33].

In mild case group, mild lung opacity was recorded with no major pathological lung changes appearance on follow up reaching complete resolution stage where fibrous stripes appear after 1 month.

CONCLUSIONS

The novel coronavirus mainly acts on lymphocytes, especially T cells, T cell types analysis especially easinophils and lymphocytes can help in early diagnosis of cases.

Cytokines level monitoring is of great benefit in early screening, diagnosis, and classification of patients before clinical symptoms and complications arouse where prognostic features will be available for future patients outcomes whether complete cure or ICU admission will be required based on his cytokine profiling system.

Acknowledgment:

I would like to express my special thanks of gratitude to my collegue Saber Nahl, Tanta University pharmacist, as well as Kamal Emam, Medical Nurse at Cairo University Hospitals who gave me the golden opportunity to do this research on COVID-19 topic which is a major health problem that is transforming the world value chains.

Secondly i would also like to thank my parents and friends who helped me a lot in finalizing this research within the limited time frame.

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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