University of Basrah
College of Pharmacy
Dep. Of Clinical Laboratory Sciences

lymphopenia and elevated inflammatory markers in Covid-19 patients

written by

نور الهدى فائق نصر
ساره مرتضى عبد الحسين
نور صباح عبد الرضا

باشراف
د.ايمان طارق علي

INTRODUCTION

- Lymphopenia as a major immunological abnormality is observed in up to 96.1% of severe COVID-19 patients, and its degrees correlate with disease outcome.[Qin, 2020]. The innate immune response to respiratory infection is characterized by an influx of neutrophils to the lungs, particularly the alveoli.[Wang Y,2018][8]. Lymphopenia, defined as a lymphocyte count less than $1x10^9$ /L (Normal range 1-3 $1x10^9$ /L) has been associated with severe COVID-19 illness [Huang H,2020; Yang X,2020 and Ruan Q,2020]; it has also been noted that patients who have died of COVID-19 had significantly lower lymphocyte counts than those who recovered [Ruan Q,2020].
- Presentations of coronavirus disease 2019 (COVID-19) range from asymptomatic to severe pneumonia with respiratory failure that can lead to invasive mechanical ventilation and/or death [Guan ,2019; Huang ,2020].
- In patients with COVID-19 and severe lung disease, hyper inflammation is frequently observed, with an increased plasma concentrations of various proinflammatory cytokines, including interleukin (IL)-6, and acute phase reactants [Qin ,2020; Chen ,2019 and Cao,2020].
- This exaggerated inflammatory response caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) represents one of the most important negative prognostic factors in these patients[Zeng *et al*.2020] .

INTRODUCTION

- **Various biomarkers are currently under investigation for their role in determination of prognosis in patients with COVID-19.**
- Lactate dehydrogenase (LDH) is one such biomarker of interest, especially since elevate LDH levels have been associated with worse outcomes in patients with other viral infections in the past . Early data in COVID-19 patients has suggested significant differences in LDH levels between patients and without severe disease.[Henry B,2020]
- . Furthermore, the other factors are C-reactive protein (CRP)&Erythrocyte sedimentation rate (ESR) were an important index for the diagnosis and assessment of severe pulmonary infectious diseases[L. Wang,2020].
- Serum ferritin, a feature of hemp phagocytes lymphohistiocytosis, which is a known complication of viral infection, is closely related to poor recovery of COVID-19 patients, and those with impaired lung lesion are more likely to have increased ferritin levels[Zhou F,2020; Fu S,2020 and Mehta P,2020].
- Evidence shows that the levels of serum ferritin, d-dimer, lactate dehydrogenase, and Interleukin-6 (IL-6)are increased during the worsening of the disease, providing an indication of the risk of mortality[Zhou F,2020].
- Thus, as a pro-inflammatory factor in the uncontrolled cytokine storm, the predictive role of the ferritin level in the risk of poor outcome in COVID-19 patients requires further verification
- Whether lower lymphocyte count and lymphopenia, inflammatory cytokines and biomarkers, including C-reactive protein (CRP), D-dimer, ferritin, ESR, could really be predictor of severity of COVID-19 were our main interest, since this laboratory tools are readily available even in the remote areas.

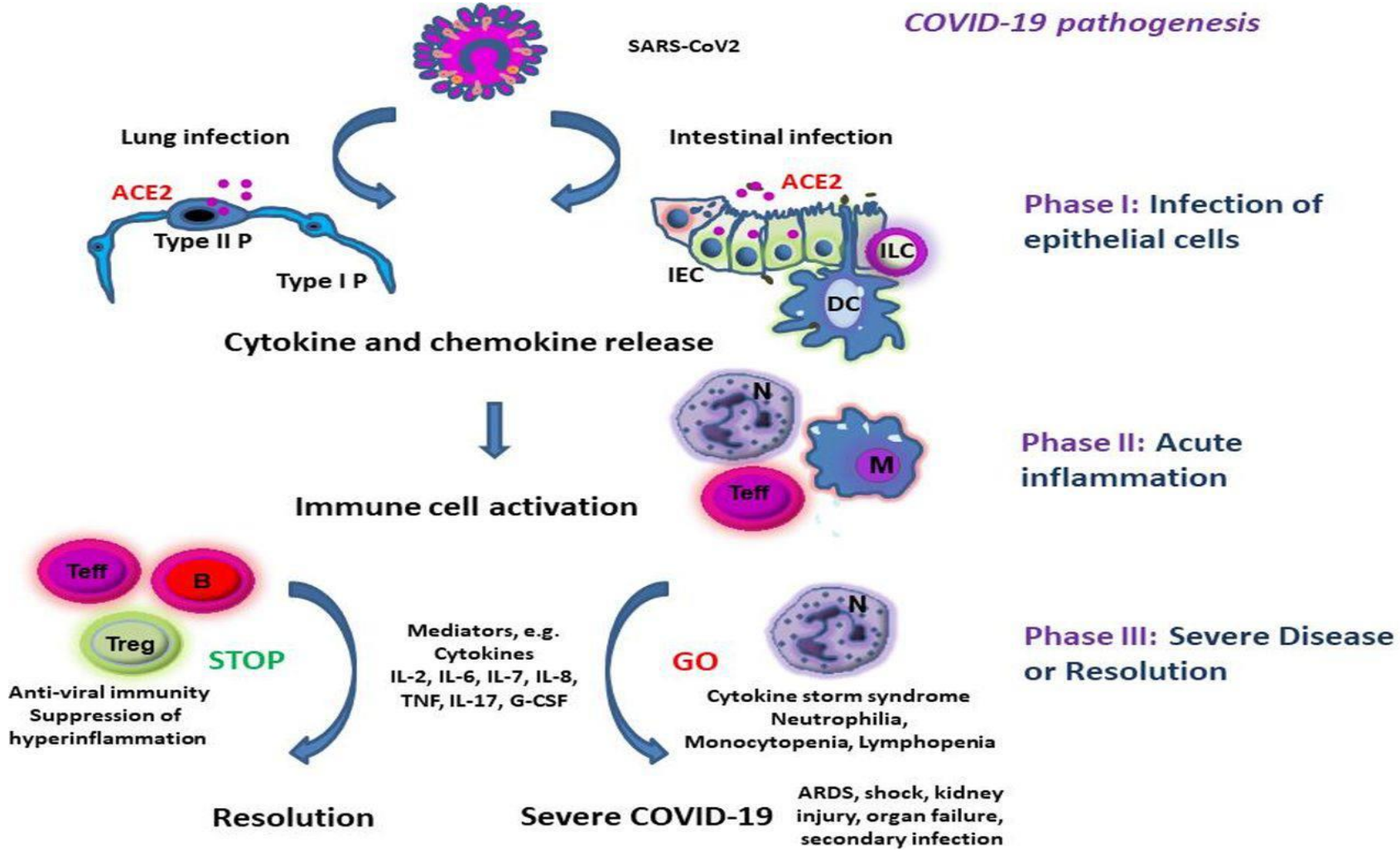


Figure1. Hypothetical pathogenesis of COVID 19.

(B, B lymphocytes; IEC, intestinal epithelial cell; ILC, innate lymphoid cell; M, monocyte/macrophage; N, neutrophils; T eff , effector T cells; Treg, regulatory T cell; Type I P, type I pneumocytes; Type II P, type II pneumocytes).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) infects ACE2 expressing epithelial cells in the lung and/or the intestine. This is followed by production of mediators causing immune cell activation. Overwhelming immune cell activation may lead to severe complications including acute respiratory distress syndrome (ARDS), shock and kidney or multiorgan failure.

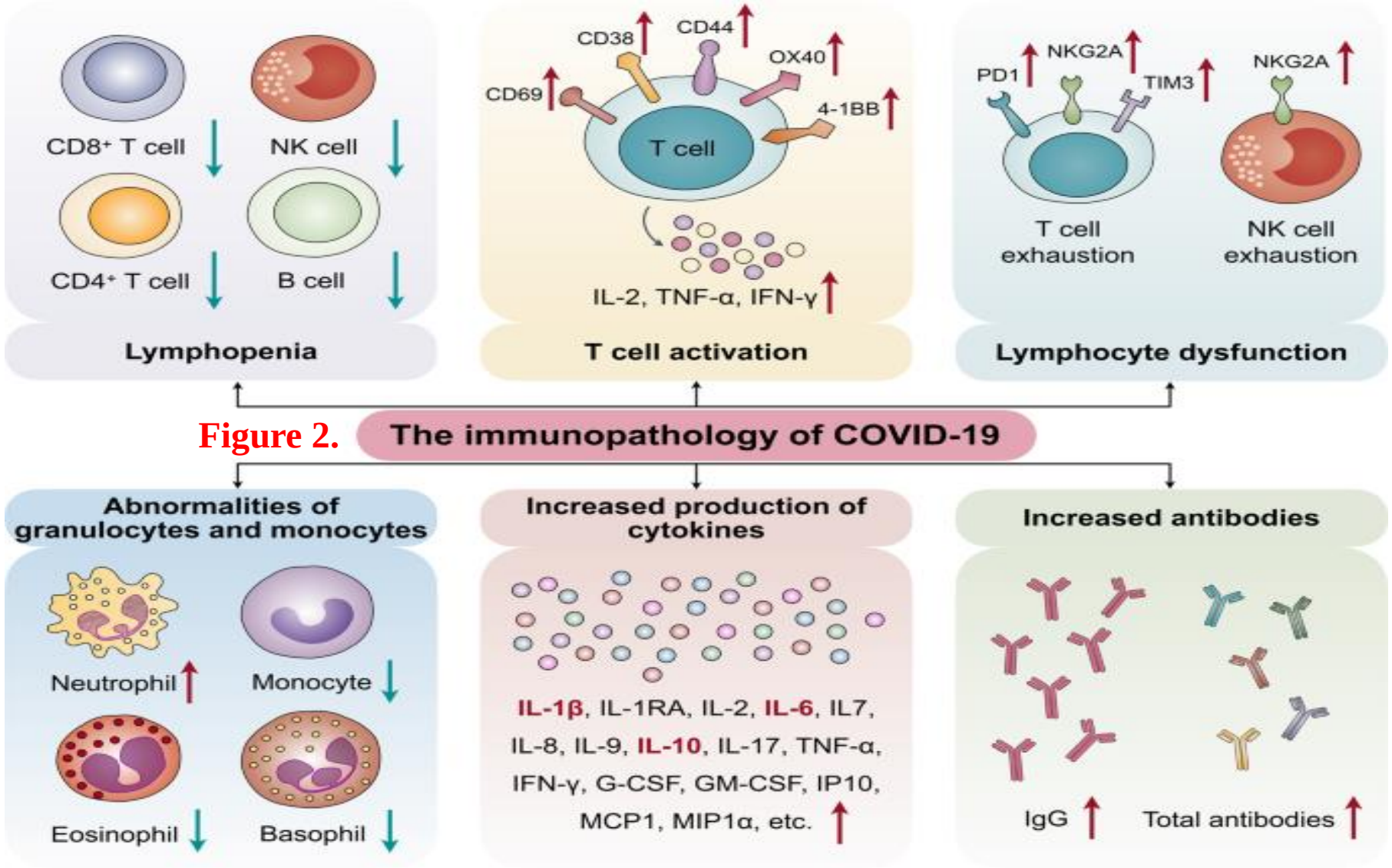


Figure 2.

It has been shown that SARS-CoV-2 disrupts normal immune responses, leading to an impaired immune system and uncontrolled inflammatory responses in severe and critical patients with COVID-19. These patients exhibit lymphopenia, lymphocyte activation and dysfunction, granulocyte and monocyte abnormalities, high cytokine levels, and an increase in immunoglobulin G (IgG) and total antibodies. The immune patterns of COVID-19 are outlined in detail in the following sections .

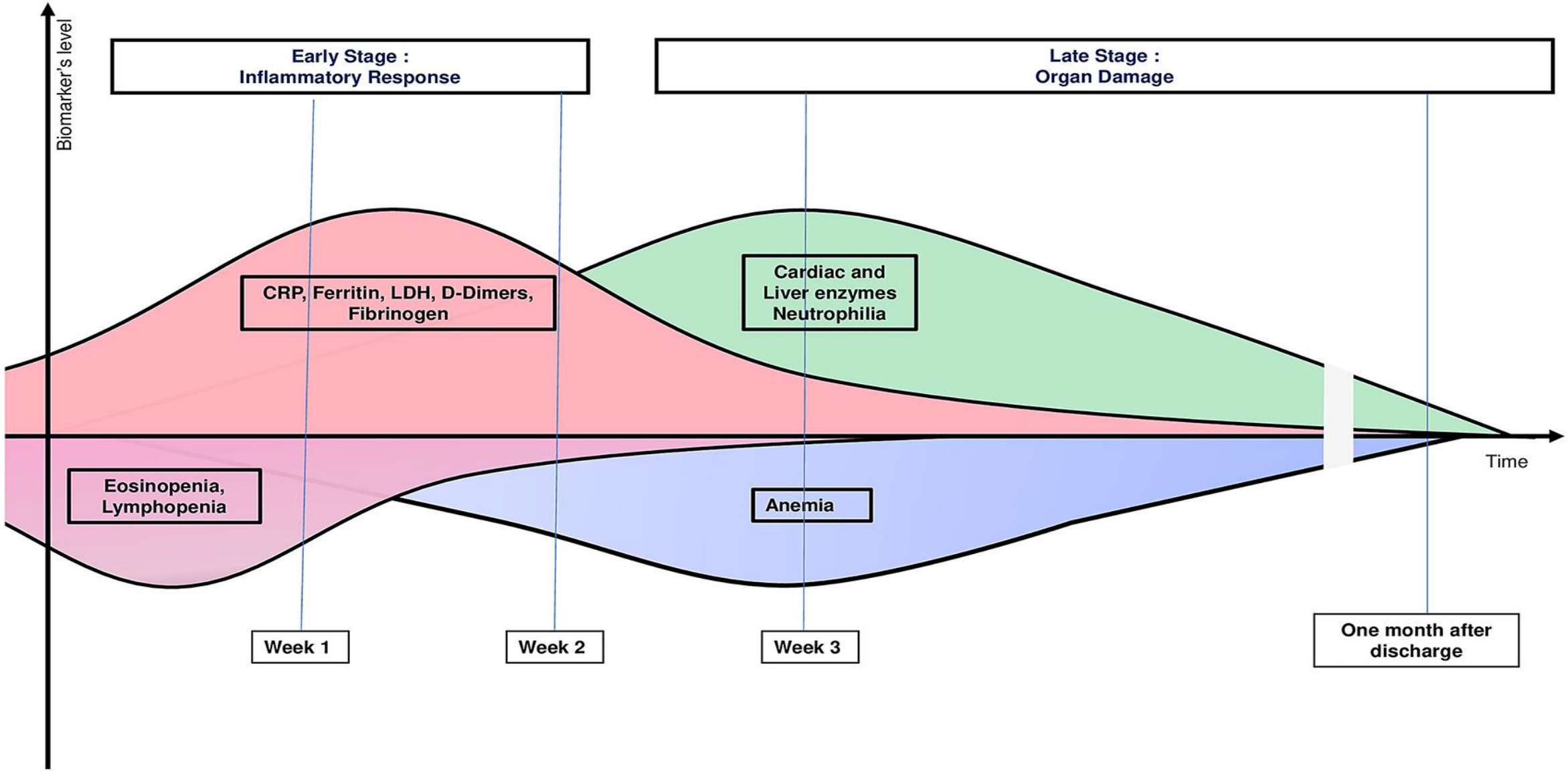


Figure 3. A biological profile for diagnosis and outcome of COVID-19 patients

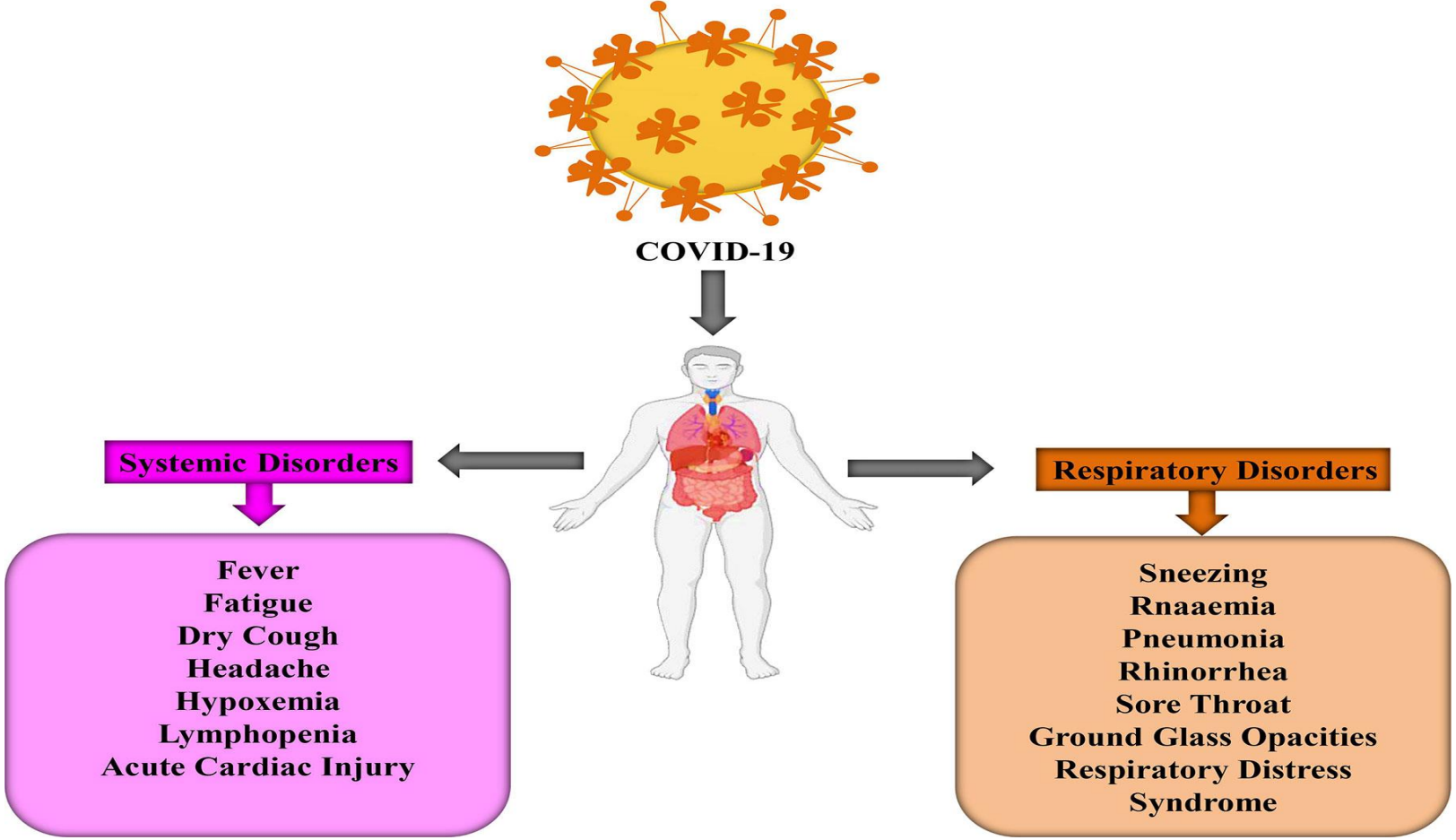
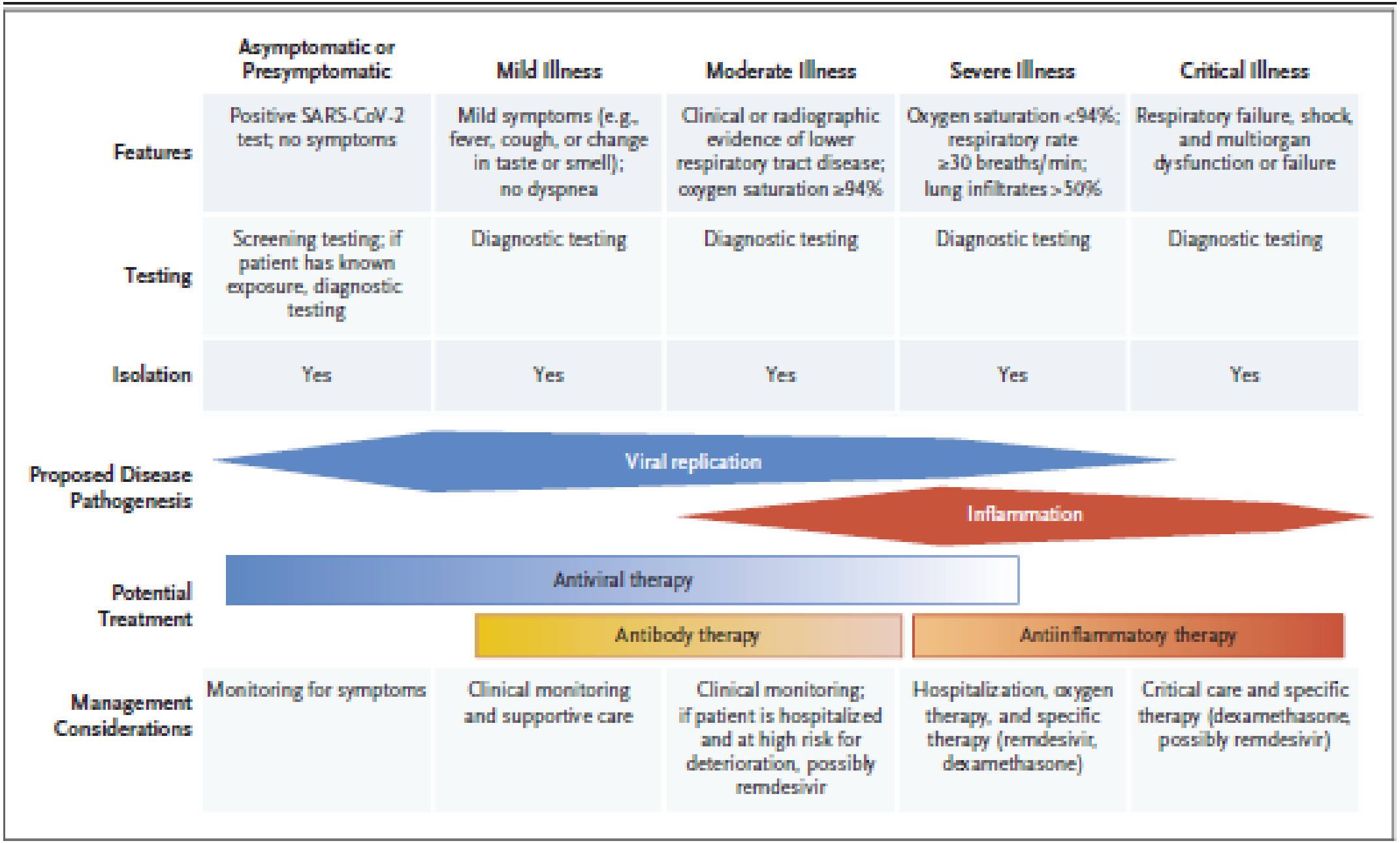


Figure 4. The systemic and respiratory disorders in COVID 19 infected patients.

COVID-19 infected people may arise their symptoms within 2 to 14 days but sometimes, in some cases, this disease prevails after 27 days. However, a group of Chinese researchers revealed that the average incubation time is approximately 5.2 days (Li Q. et al., 2020). During this period, there is no significant change in peripheral blood leukocytes (PBL) and lymphocytes. Generally, the viruses spread out in the lungs, heart, GIT, and through the bloodstream. Primary lesions become noticeably worse around 7–14 days and PBL reduces significantly, including both T and B-lymphocytes (Li T. et al., 2020). The time frame for COVID-19 patients' symptoms to death has been observed 6 to 41 days where the median period is 14 days (Wang W. et al., 2020). Although this period depends on two crucial factors, namely, the patient's age and immune function.


Asymptomatic or Presymptomatic
Mild Illness
Moderate Illness
Severe Illness
Critical Illness
Features
Positive SARS-CoV-2 test; no symptoms
Mild symptoms (e.g., fever, cough, or change in taste or smell); no dyspnea
Clinical or radiographic evidence of lower respiratory tract disease; oxygen saturation ≥94%
Oxygen saturation <94%; respiratory rate ≥30 breaths/min; lung infiltrates >50%
Respiratory failure, shock, and multiorgan dysfunction or failure
Testing
Screening testing; if patient has known exposure, diagnostic testing
Diagnostic testing
Diagnostic testing
Diagnostic testing
Diagnostic testing
Isolation
Yes
Yes
Yes
Yes
Yes
Proposed Disease Pathogenesis
Viral replication
Inflammation
Potential Treatment
Antiviral therapy
Antibody therapy
Antiinflammatory therapy
Management Considerations
Monitoring for symptoms
Clinical monitoring and supportive care
Clinical monitoring; if patient is hospitalized and at high risk for deterioration, possibly remdesivir
Hospitalization, oxygen therapy, and specific therapy (remdesivir, dexamethasone)
Critical care and specific therapy (dexamethasone, possibly remdesivir)

Aim of the study

- **The aim of this study was to identify**
- **Whether lower lymphocyte count and inflammatory biomarkers, including LDH, C-reactive protein (CRP), D-dimer, ferritin, ESR, could really be predictor of severity of COVID-19 were our main interest, since this laboratory tools are readily available even in the remote areas.**

Materials and Method

- ***Study Design*** **and participants**
- The study was conducted in Basra City, South of Iraq, All patients were admitted from April 17, 2020, to February 14, 2021. The diagnosis was based on clinical criteria and laboratory features according to WHO interim guidance [7]. The final date of follow-up was March 13, 2021.Among 194 patients with COVID-19 registered in the database, we first excluded seven patients diagnosed with COVID-19 after death, 27 patients with no COVID-19 course data, and 116 patients without baseline data of complete blood counts. For the total cohort consists of 44 patients having baseline data, we investigated those whose factors associated with lymphopenia.
- **Data collection**
- In all patients, infection with SARS-CoV-2 was confirmed by real-time fluorescent reverse transcription PCR detection of virus-specific nucleic acid in sputum and throat swab specimens, chest radiographs and computed tomographic (CT) scans were performed in the initial days after admission .
- Epidemiological and clinical data were collected from COVID-19 patients upon hospitalization. Illness severity was defined according to the Chinese management guidelines for COVID-19 (version 7.0).
- . Blood count and serum values of creatinine, lactate dehydrogenase (LDH), C-reactive protein (CRP) were quantified in all patients enrolled in the study. Serum samples were analyzed on a fully automated clinical chemistry Instrument (Beckman Olympus, Beckman, Germany)..

Materials and Method

- 2.3. ***Sample collection***. After obtaining approval from the participants orally, five milliliters of blood was drawn from each worker using a vein puncture approach and then collected in two tubes. The rest of the blood was contained in a gel plain tube to prepare the serum for further study. To avoid any effects of ambient temperatures on the samples, the collected blood samples were always stored in cold conditions with a wet icebox until the time of measurement. Samples were measured on the same day. All laboratory work was performed in a private laboratory for analyzes

- 2.4. ***Hematology Profile***. Two ml of venous blood in a tube containing EDTA (disodium ethyl nediam inetetraacetate) have been used to measure the haematological indices.

- **2.4.1.*Complete Blood Count* (CBC**) Test. Hematological parameters making up the Complete Blood Count (CBC) i.e (RBC, HGB g/dl, MCV(fl), MCHC(g/L), MCH(pg), total White Blood Cell count and differential count (lymphocyte, monocytes and neutrophils) were performed using the Auto Hematology Analyzer (Ruby, Germany). According to the WHO criteria, workers with hemoglobin value <13 g/dL in men were diagnosed anemic .

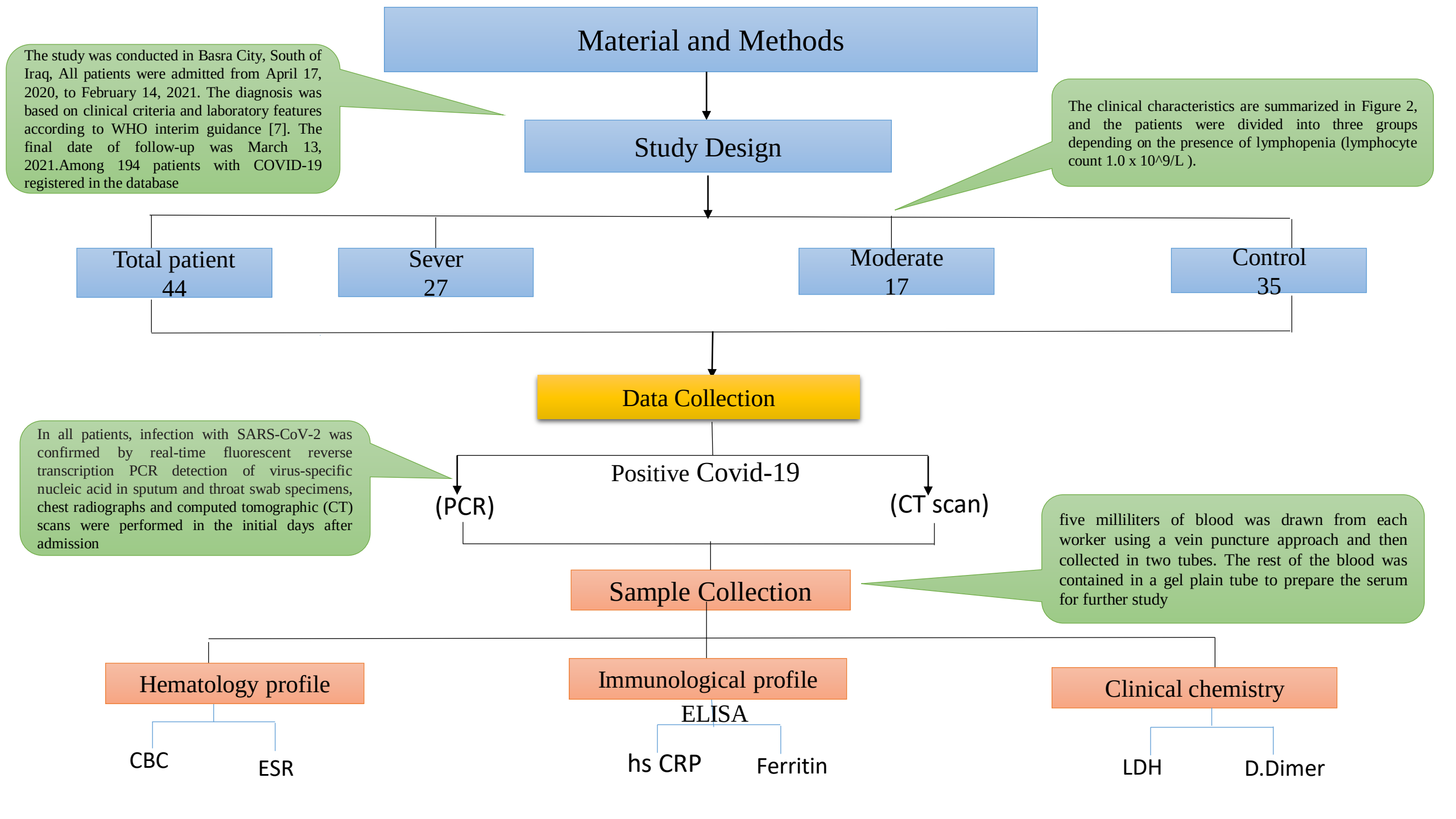
- 2.4.2. ***Erythrocytes Sedimentation Rate* (ESR**) Test. Non-specific body-screening test Erythrocytes Sedimentation Rate (ESR) was measured by the Westergren method the unit being millimeter per hour (mm/h)[13].

- 2.5.2. ***Evaluation of (hs-CRP) protein Level*** was estimated by (ELISA).

- 2.5.3.The quantitative measurement of human ferritin concentration was estimated by (ELISA) according to the specifications provided by the manufacturer (POINTE scientific, Inc, USA)

Materials and Method

- 2.6**. *Statistical Analysis***. Statistical analysis of the data was done using Statistical Package for the Social Sciences (SPSS) software for Windows, version 24.0, IBM (SPSS Inc., IL, USA). The data is represented as an average value ± standard deviation (SD). The comparison of two sets of differences in the distributed numerical variables was estimated by Independent students' -test (Groups 1 and 2)..Pearson correlation test was used to find out the correlations between different laboratory findings .The significance level was measured by two-tailed paired. P-values at levels ($p<0.05$) were significant


Material and Methods
The study was conducted in Basra City, South of Iraq, All patients were admitted from April 17, 2020, to February 14, 2021. The diagnosis was based on clinical criteria and laboratory features according to WHO interim guidance [7]. The final date of follow-up was March 13, 2021.Among 194 patients with COVID-19 registered in the database
Study Design
The clinical characteristics are summarized in Figure 2, and the patients were divided into three groups depending on the presence of lymphopenia (lymphocyte count 1.0 x 10^9/L).
Total patient 44
Sever 27
Moderate 17
Control 35
Data Collection
In all patients, infection with SARS-CoV-2 was confirmed by real-time fluorescent reverse transcription PCR detection of virus-specific nucleic acid in sputum and throat swab specimens, chest radiographs and computed tomographic (CT) scans were performed in the initial days after admission
Positive Covid-19
(PCR)
(CT scan)
five milliliters of blood was drawn from each worker using a vein puncture approach and then collected in two tubes. The rest of the blood was contained in a gel plain tube to prepare the serum for further study
Sample Collection
Hematology profile
CBC
ESR
Immunological profile
ELISA
hs CRP
Ferritin
Clinical chemistry
LDH
D.Dimer

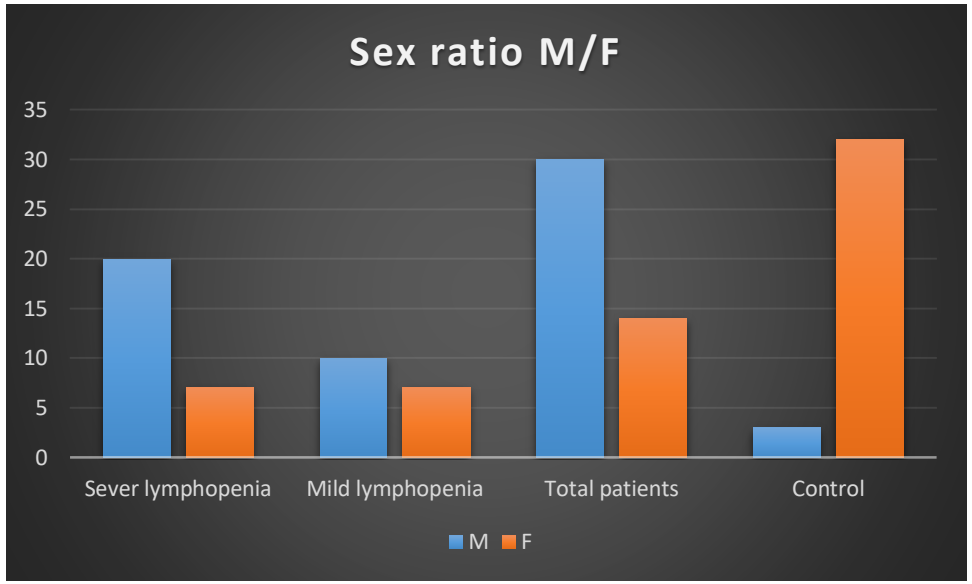
REULTS
&
DISCUSSION

Table 1:Demographic characteristics among Covid -19 patients

Characteristic	Total patients	Moderate lymphopenia	sever lymphopenia	control	p≤
Number	44	17	27	35	
Sex,F/M	14/30	7/10	7/20	32/3	
Age(year)	46±11.07	49.7±13.3	43.66±8.9	38.8±7.86	0.2
Rang	34-80	34-80	34-68	25-60	
Comorbidities	N%	N	N	N	
Diabetes	33(75)	10	23	0	
Hypertension	5(11.4)	3	2	-	
Cholesterol	6(13.6)	4	2	-	

P is significant statistics at level < 0.05.

Data are reported as mean ± standard deviation (range).



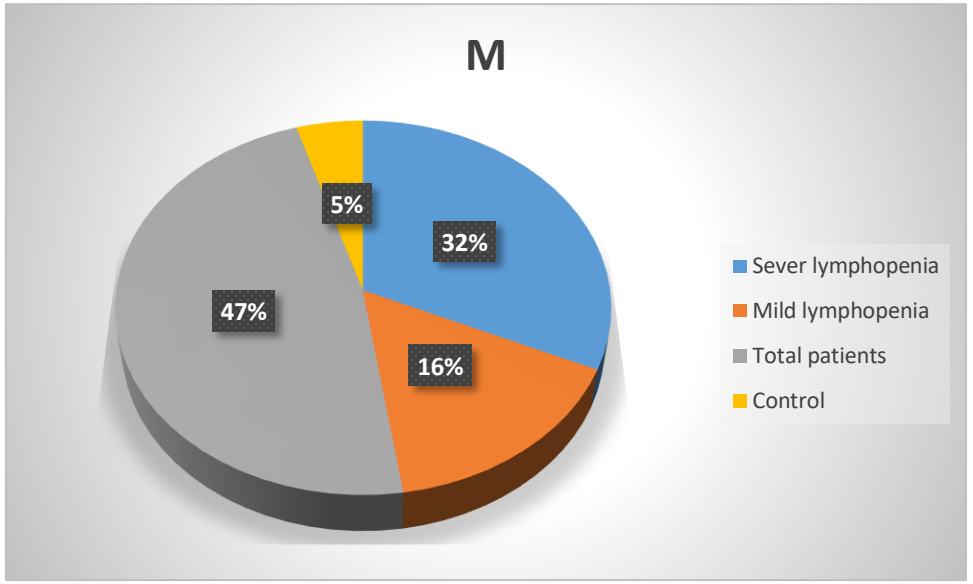
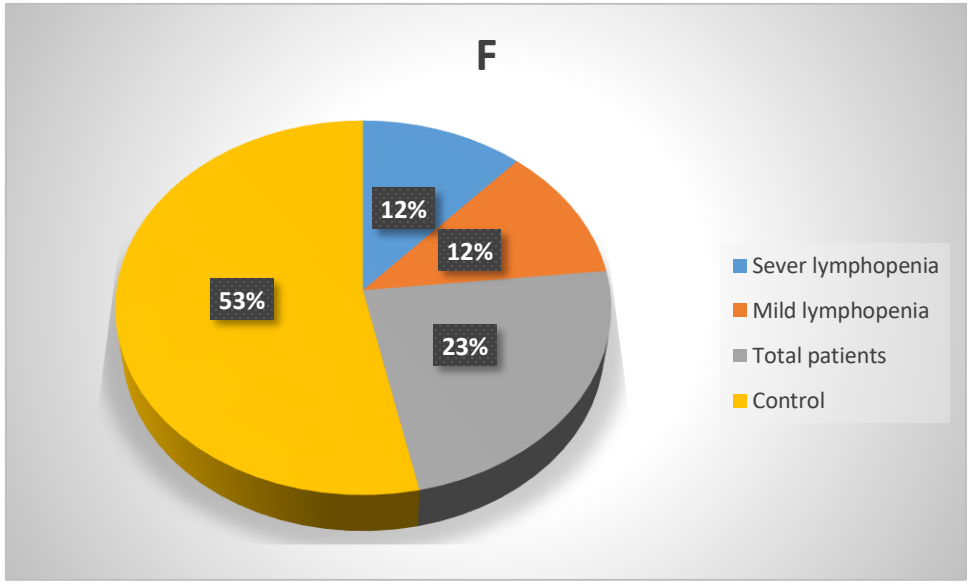
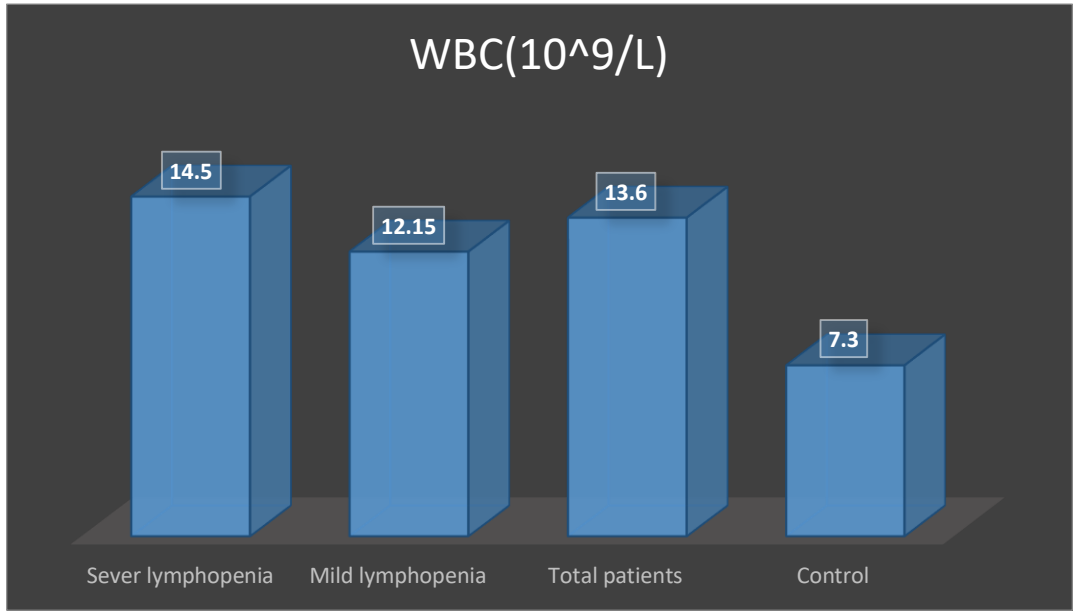


Figure 1:Percentage of males and females with Covid -19 in the current study



Results have shown that the percentage of males affected more than females infected with Covid -19 compared between the four totals

44 patients (mean age 46±11.07 years) were identified to be SARS-CoV-2 positive and 68.1% of cases were male. 17 patients had moderate severity and 27 were sever.



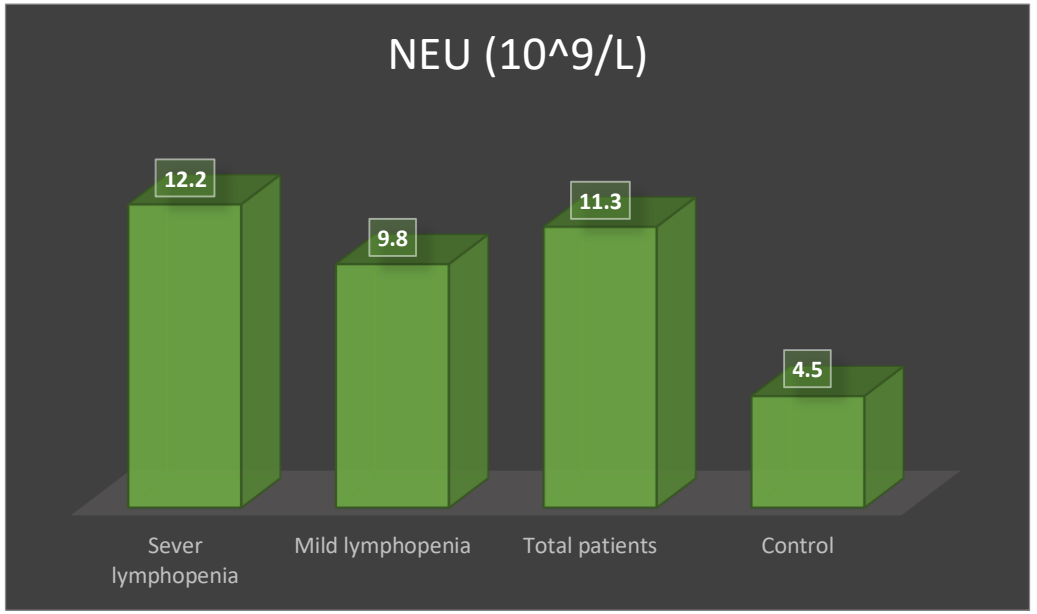


Figure 2:leukocyte and Neutrophil counts in different groups of COVID-19 patients compared to control.

Our results indicated that COVID-19 positive cases had leukocytosis with an increased number of neutrophils and decreased lymphocyte count. The median WBC &Neu count of sever cases were significantly higher than in general groups ($P<0.0001$).

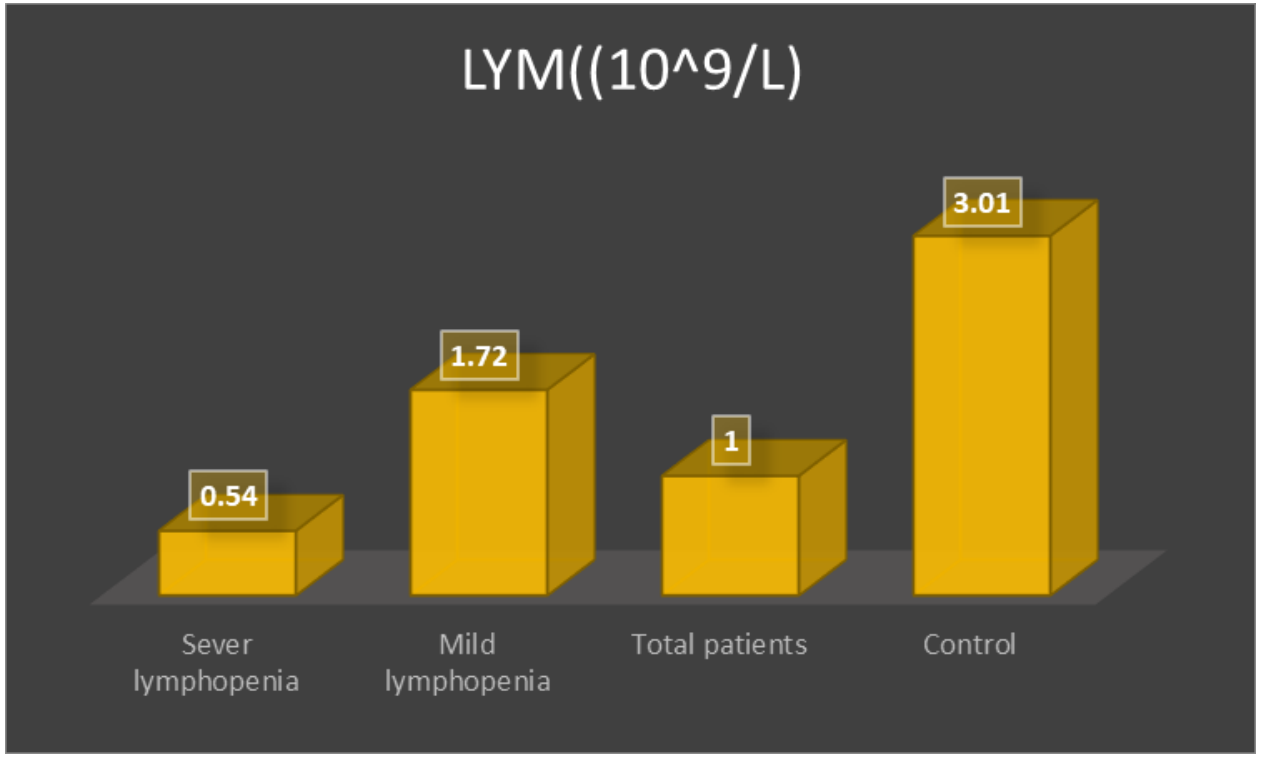
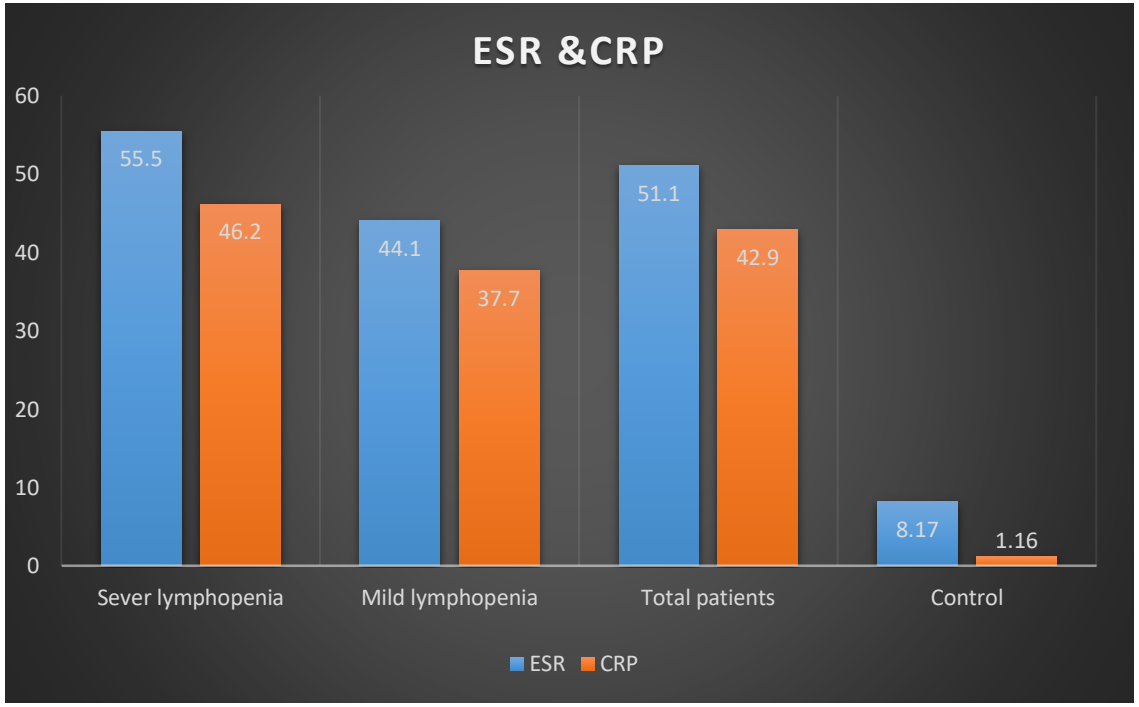


Figure 3:Lymphocyte counts in different groups of COVID-19 patients compared to control.

The median lymphocyte counts were significantly different among the four groups (P<0.0001) .The median lymphocyte count of sever cases 0.54±0.21 (10^9/L) was significantly lower than in general groups.



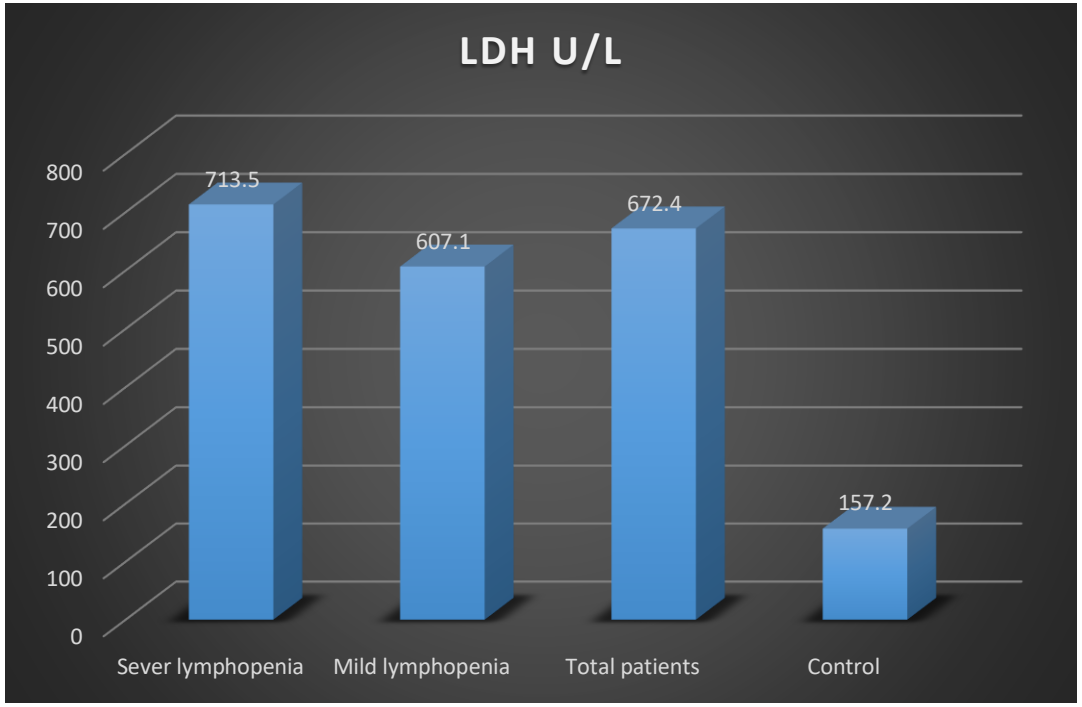


Figure4. Inflammatory markers, including CRP ,LDH and ESR values in CoVID-19 patients groups.

The results showed very high significant differences in the levels of both hsCRP and ESR in sever patients than other groups patients when compared with controls (P<0.0001). In addition , there were significant differences in LDH levels between &within groups (P<0.0001). In CoVID-19 patients, LDH and CRP might represent an expression of lung damage and might reflect the respiratory distress consequent to the abnormal inflammation status.

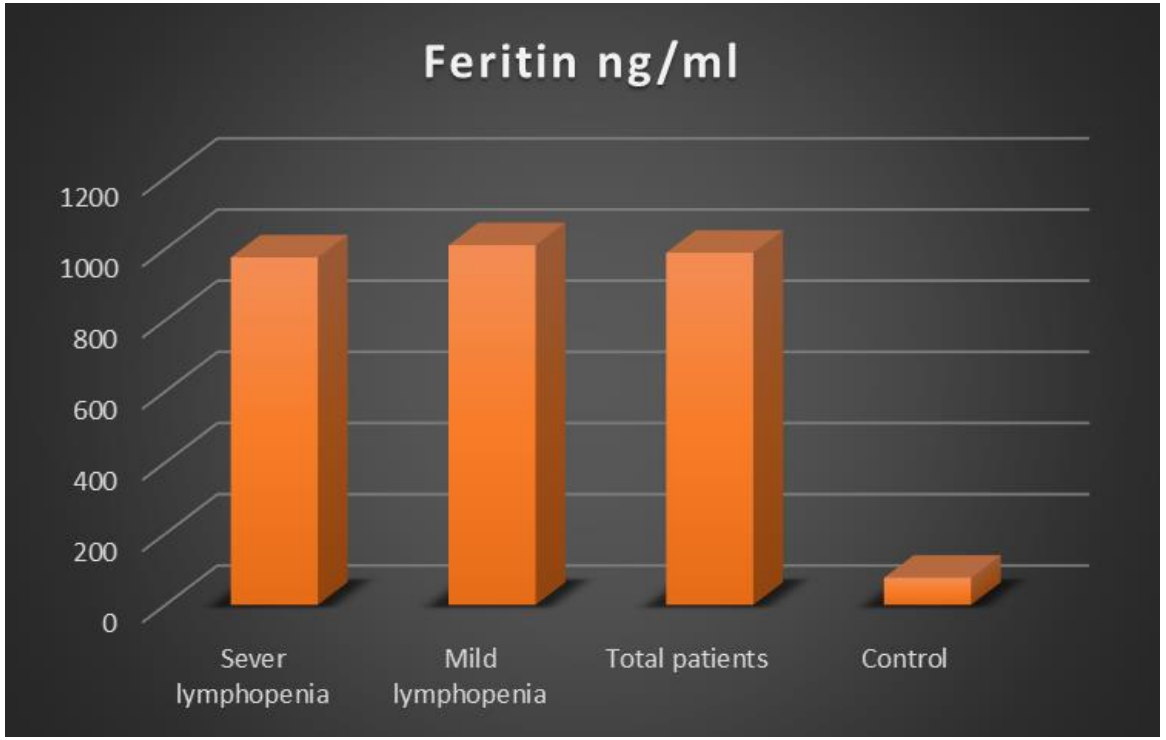
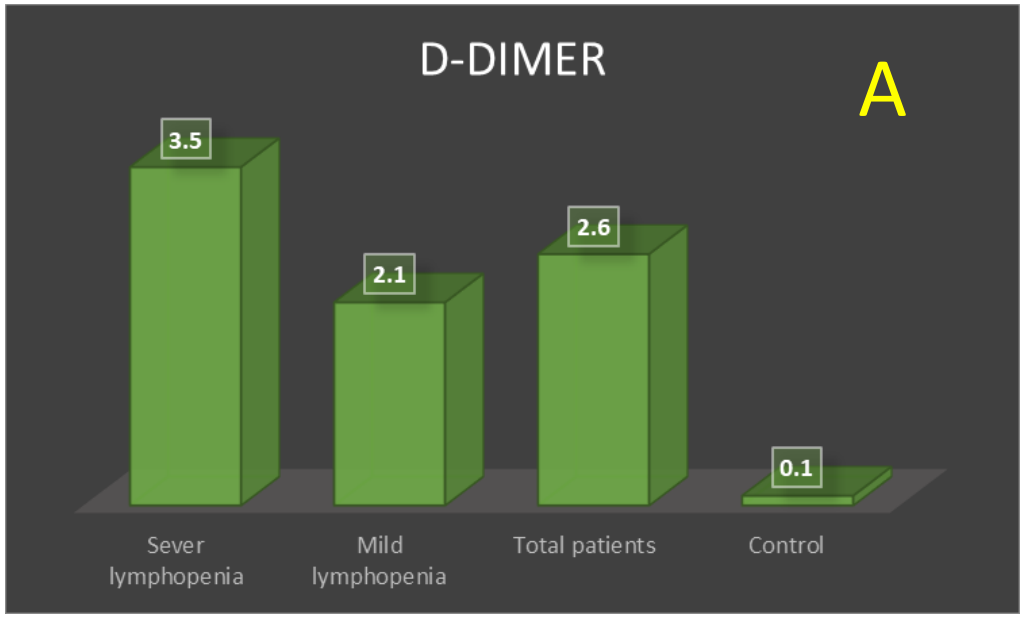


Figure 5. The level of ferritin in Covid -19 patients compared to control

The ferritin level was significantly increased in severe patients compared with the level in other groups. Statistically significant difference in ferritin was found in the three groups based on severity and mortality ($P<0.0001$)..



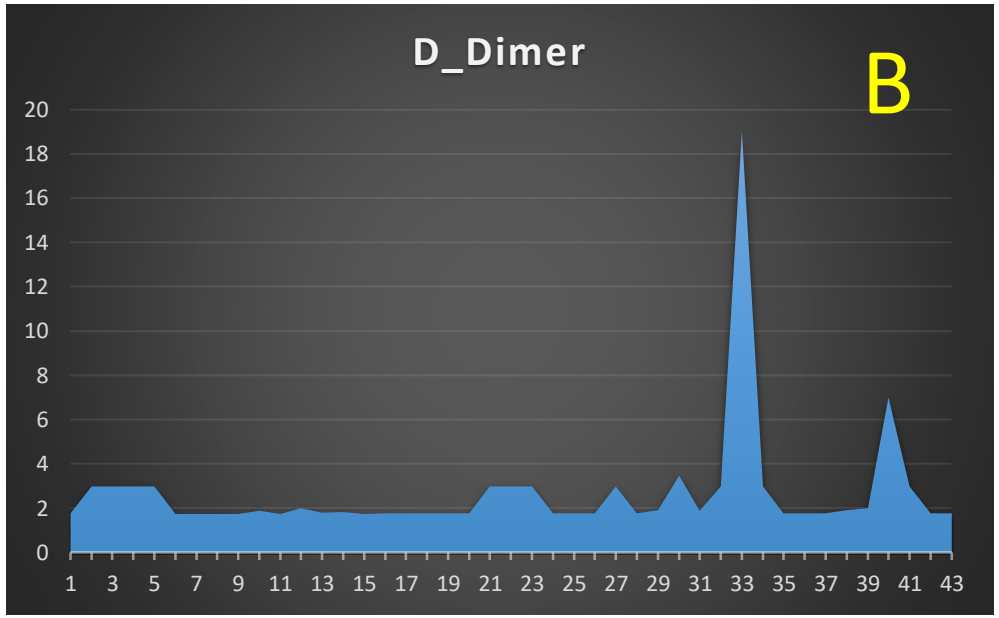


Figure 6. The levels of D-dimer in patients with COVID-19between the groups

A. The results showed that there were no significant differences between groups, but there were differences in its levels among patients ($P<0.001$).

B. Significantly higher levels are observed in those with critical illness was (19.02 ug/ml)and can be used as a predictive marker of in-hospital mortality fig B.

Conclusion

Based on our results:

- Lymphopenia an important immunological abnormality in patients with COVID-19.
- Dynamic changes in routine blood parameters might be helpful for the prognosis of COVID-19 patients and evaluation of the treatment effect.
- Based on our results, we believe that dosing LDH and CRP could be useful to the early identification of patients at high risk for acute respiratory failure, even in patients who do not complain dyspnea or affected by slight respiratory failure.
- 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 for COVID-19.
- Ferritin was associated with poor prognosis and could predict the worsening of COVID-19 patients


Thank
you