Research Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2023.49.007737

New Parameters in Covid-19 Therapy Guidance: A Retrospec-Tive Study

Michela Pontolillo[1], Katia Falasca[1]*, Paola Borrelli[2], Myriam Luongo[3], Marta Di Nicola[2], Jacopo Vecchiet[1] and Ucciferri Claudio[1]

[1]*Clinic of Infectious Diseases – Department of Medicine and Science of Aging, University "G. d'Annunzio" Chieti-Pescara, Italy*

[2]*Laboratory of Biostatistics, Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio", Chieti- Pescara, Italy*

[3]*Clinical pathology, SS Annunziata Hospital, Chieti PB, Italy*

***Corresponding author:** Katia Falasca, Clinic of Infectious Diseases, Dept. of Medicine and Science of Aging, "G. d'Annunzio" University, School of Medicine, Via dei Vestini, 66013 Chieti, Italy

ARTICLE INFO

Received: February 10, 2023

Published: March 01, 2023

Citation: Michela Pontolillo, Katia Falasca, Paola Borrelli, Myriam Luongo, Marta Di Nicola, Jacopo Vecchiet and Ucciferri Claudio. New Parameters in Covid-19 Therapy Guidance: A Retrospec-Tive Study. Biomed J Sci & Tech Res 49(1)-2023. BJSTR. MS.ID.007737.

ABSTRACT

Objectives: Laboratory examinations with new parameters can help the clinician through the path of care of COVID-19 patient. The aims of this study to evaluate the relationship between the severity of the disease, the qualitative and hemocytometric data obtained within three days of hospital admission; and between the latter and the stage of the disease described by the patient.

Materials and Methods: This retrospective, single-center cohort study included patients with COVID-19 diagnosis, from April 26, 2021, to October 24, 2021. 68 patients were enrolled, and they did a complete blood count with positional parameters, immature granulocytes and their relationship to lymphocytes and all these data were collected in three stages in the first three days of hospitalization.

Results: The relationship between neutrophils and lymphocytes (NRL) showed a progressive in-crease over time and in particular statistically higher levels between moderate and mild case and between case severe and moderate ($p< 0.001$). The Delta-He and Hypo-He showed statistically significant differences in the three days of hospitalization ($p< 0.001$). The IL-6, a pivotal cytokine in self-sustaining inflammatory storm, remains higher during the first three days of hospitalization in severe cases, resulting in a statistically significant difference with the other groups during the observation period($p< 0.01$). The CRP have a significant difference only on the first day, between severe and mild cases and between severe and moderate cases($p< 0.001$). Finally, the ferritin is confirmed to be higher in severe patients. It remained higher in the three days.

Conclusion: The dosing of factors such as NLR, IGLR, As-lymph/L, Delta-He, Hypo-He, IL-6, LDH, ferritin could be useful for the early identification of patients at high risk of moderate/severe acute respiratory failure. These hematochimical parameters, which are easy and quick to perform, and who may require different and more specific treatment protocols. These hematochimical parameters, which are easy and quick to perform, and who may determine the use of different and more specific treatment protocols in patients with diagnosis of COVID-19.

Keywords: COVID-19; Neutrophils; Lymphocytes; Delta-He; Hypo-He; IL-6

Abbreviations: WHO: World Health Organization; NLR: Neutrophil Lymphocytes; CRP: C-reactive Protein, PCT: Procalcitonin, LDH: Lactate Dehydrogenase; RET: Reticulocyte Count, PLT: Platelets; IG: Immature Granulocytes; IPF: Fraction of Immature Platelets; VMNI: Non-Invasive Mechanical Ventilation; HFNC: High Flow Nasal Cannula; NIV: Non Invasive Ventilation; NPPV: Non Invasive Positive Pressure Ventilation; CPAP: Continuous Positive Airway Pressure; IGRL: Absolute Number Of Lymphocytes; NLR: Neutrophils To Lymphocytes

Introduction

COVID-19 can present with a wide range of clinical manifestations, from asymptomatic pneumonia to a severe form with multiple organ failure [1], threatening global health. Early identification of critically ill patients can reduce mortality through timely interventions [2]. The mitochondrial dysfunction in COVID-19 continues to be studied, the use of mt-DNA as an indicator of prognosis and severity is a potential area yet to be explored [3] and there are different studies the needs and available tools for clinical-physiological monitor-ing that aims at optimizing the ventilatory management of patients affected by acute respiratory distress syndrome due to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection [4]. According to Siddiqui and Mehra [5], SARS-coV-2 disease begins as a viral infection and evolves into a self-sustained hyper inflammatory response. We can therefore distinguish three phases. A first viremic phase (from 1st to 6th day of symptoms), in which SARS-coV-2, after having penetrated into the host's cells, replicates and the patient begins to develop symptoms. A second inflammatory phase (7-10 days), characterized by morpho-functional alterations in the lungs caused both by the cytopathic effects of the virus and by the host's immune response. A third phase of Cytokine Storm is lung damage (> 11 days), where the hyperactive inflammatory state determines local and systemic consequences that can lead to severe and sometimes permanent lung injury [6].

The latest generation hemocytometers, thanks to the technological evolution characterized by the introduction of new physical principles for cellular analysis and a progressive development of management «software», have made available new quantitative and qualitative hematological parameters, useful for the characterization of peripheral blood cells. The use of these parameters can be useful both in the formulation of a diagnostic hypothesis and in the monitoring of different pathological pictures [7,8]. In particular, leukocyte parameters have been found to be useful in infectious diseases, expression of the dimensional and morpho-functional variations that leukocytes develop during infection [9-11]. In fact, clinicians need to rely on valuable methods to identify critical cases of COVID-19. Laboratory examinations with new parameters can help the clinician through the path of care of COVID-19 patient. With these backgrounds, we designed this study to evaluate the relationship between the severity of the disease, the qualitative and hemocytometric data obtained within three days of hospital admission; and between the latter and the stage of the disease described by the patient. The goal is to identify useful parameters in the stratification of the risk of worsening, then recognize the clinical phenotype of patients who should are expected to have moderate and severe disease, through hematochimical parameters, which are easy and quick to per-form, in order to allow early therapeutic choices that improve the patient's prognosis.

Materials and Methods

Study Design and Population

This retrospective, single-center cohort study included patients with COVID-19 admitted to the Infectious Diseases Clinic of the University Hospital of Chieti in Abruzzo, Central Italy, from April 26, 2021, to October 24, 2021. The study was conducted in compliance with the principles embodied in the Declaration of Helsinki and all participants provided written informed consent. This study was approved by the internal hospital ethics committee and informed consent was waived due to the observational nature of the study. All patients were diagnosed with COVID-19 according to World Health Organization (WHO) guidelines: presence of clinical symptoms of COVID-19 and confirmation of SARS-CoV-2 infection through a positive result on RT tests -PCR of nasopharyngeal and oropharyngeal swab specimens.

The inclusion criteria were as follows:

- Patients confirmed by positive detection of SARS-CoV-2 RNA from nasopharyngeal/pharyngeal swabs by RT-PCR with clinical symptoms suggestive of COVID-19;
- Patients over 18 years of age;
- Need for hospitalization for respiratory failure.
- Exclusion criteria:
- Age <18 years;
- Pregnant women;
- Hospitalization for non-COVID-19 related problems in a patient with a positive swab;
- Hematological diseases;
- Recent blood transfusions.

Epidemiological, demographic, clinical, laboratory, treatment and outcome data were extracted from medical records using a standardized data collection form.

Data Collecting

They consisted in a complete blood count with positional parameters, immature granulocytes and their relationship to lymphocytes (IGLR), reticulocytes, activated lymphocytes (Re-lymph, As-lymph), Delta-He, Hypo-He, ratio neutrophil lymphocytes (NLR), coagulation test, liver function assessment, calcium, C-reactive protein (CRP), procalcitonin (PCT), lactate dehydrogenase (LDH), ferritin, and IL-6. All these data were collected in three stages in the first three days of hospitalization. The haemocytometric examination was performed with a Sysmex XN analyzer, which, by integrating traditional optical and impedance measurement techniques with fluorescence flow cytometry. It is able to provide, in addition to the standard blood count parameters, additional reportable parameters too, as : reticulocyte count (RET), platelets (PLT) with the optical fluorescence method (PLT-F), count of erythroblasts (NRBC), immature granulocytes (IG) and the fraction of immature platelets (IPF), determination of the amplitude of the distribution curve of red

blood cells (RDW-CV or RDW-SD) and platelets (PDW), plateletcrit (PCT), mean reticulocyte volume (RET-He). Lymphocytopenia was defined as a lymphocyte count of less than 1100 cells per cubic millimeter, and thrombocytopenia as a platelet count of less than 150,000 per cubic millimeter [12].

Among the leukocyte positional parameters, mainly those of lymphocyte activation were taken into consideration: Re-lymph, As-lymph. Re-lymph stands for reactive lymphocytes and reflects all lymphocytes that have a higher fluorescence signal than the normal lymphocyte population. The As-lymph population is always included in Re-lymph count. This parameter quantifies the activated B-lymphocytes (plasma cells) that synthesize antibodies. The Delta-He parameter reflects the difference between the hemoglobin equivalent of reticulocytes (RET-He) and the hemoglobin content of mature red blood cells (RBC-He). Under physiological conditions, the Delta-He value is positive since the hemoglobin content in reticulocytes is higher than that of mature red blood cells. The Delta-He parameter can measure iron deficiency in a highly sensitive and accurate way. Hypo-He is the percentage of RBCs with cellular hemoglobin content less than 17 pg. The neutrophil to lymphocyte ratio (NLR), easily calculated from a routine blood test by dividing the absolute neutrophil count by the absolute lymphocyte count, is of great value in indicating a patient's overall inflammatory status.

We divided patients in three phases of diseases, in according to WHO. A first viremic phase, a second inflammatory or transition phase and a third phase of Cytokine Storm is lung damage [13].

The classification of the patient into severity classes was done by assessing the need for oxygen therapy and the type of support used to carry it out. So, we could divide our patients as follows:

- Mild CASE when there was a need for oxygen therapy in the nose cannulae or Venturi mask (VMK) (pO2 25-60%);
- MODERATE CASE need for Non Invasive Mechanical Ventilation (VMNI) using High Flow Nasal Cannula (HFNC), Non Invasive Ventilation (NIV) o Non Invasive Positive Pressure Ventilation (NPPV), Continuous Positive Airway Pressure (CPAP) (with need PEEP);
- SERIOUS CASE, need for transfer to the Intensive Care Unit (ICU) (with P/F< 100).

Statistical Analysis

Descriptive analysis was carried out using median and interquartile range (IQR) for the quantitative variables and percentages values for the qualitative ones. Normality distribution for quantitative variables was assessed by the Shapiro-Wilk Test. Pearson's chi-square test or Fisher's exact test was used to evaluate the association between categorical variables while the non-parametric Kruskal-Wallis test to evaluate the differences between continuous variables and outcome considered. After the Kruskal Wallis test, for the statistically significant results, the Dunn test was calculated for the comparison between the pairs of medians for the identification of significant differences. The Bonferroni's correction for multiple comparisons tests was applied. Statistical significance was set at the level of ≤0.05, unless adjustment for multiple comparisons was needed (in this case the significance threshold was 0.0167 (p/k, assuming k = 3 contrast)). All analyses were performed using Stata software v15.1 (StataCorp, College Station, USA).

Results

68 patients were enrolled within the cohort. One patient, who entered due to metrorrhagia and severe anemia with the need for blood transfusions, was subsequently excluded, as the blood count was not usable. The mean age was 59±16.7 years (range 29-90) and 62.7% of patients were male. Upon admission to the emergency room, patients reported fever (86.6%), as the predominant symptom, followed by cough (74.6%), asthenia (55.2%), dyspnea (40.3%), myalgia (32.8%); diarrhea/vomiting (17.9%), pharyngodynia (14.9%), ageusia (9.1%), anosmia (4.5%). Hypertension was present in 39 patients (57.4%), 12 patients had diabetes (17.6%), 11 patients had ischemic heart disease (16.2%), and 7 had heart failure (10.3%) in history. Chronic obstructive pulmonary disease was present in 8 patients (11.8%), while asthma in 3 patients (4.4%). 7 patients (10.3%) had atrial fibrillation, 3 stroke outcomes (4.4%), 3 unspecified arrhythmias (4.4%), 2 pulmonary embolism (2.9%). 5 patients had chronic renal failure (7.4%), while 2 had non-hematological active neoplasm (3%). Furthermore, 25 patients were obese (36.8%).

Regarding pre-infection vaccination coverage, only 12 patients (17.95%) were vaccinated with a full course (88.3% with mRNA vaccine). Additionally, 18 patients (27.3%) received corticosteroid therapy at home after COVID-19 diagnosis. The CT findings were classified as follows: 34.3% had bilateral pneumonia mainly with ground glass, 29.9% had a GGO picture with thickening of the septa, 35.8% had consolidations. All patients underwent therapy according to the guidelines in force during the hospitalization period: 59.7% of patients received Remdesivir, 37.3% Dexamethasone, 2.9% Anti-Spike monoclonal anti-bodies. From the medical records it emerged that 50% of patients did not respond significantly to the first line of therapy set (need for increased oxygen enrichment), so the clinicians needed to set up a second line of therapy. This was made up of 30.9% dexamethasone, while 8.8% of an anti-inflammatory anti-interleukin drug. 86.8% of patients received prophylactic Low Molecular Weight Heparin (EPBM), and 11.8% of patients were already on anticoagulant therapy by TAO/NOAC (new oral anti-coagulants). The 92.5% (62) of patients recovered with subsequent home discharge and 7.5% (5) of patients died; during hospitalization 13 patients (19.1%) required transfer to ICU. The patients of our co-hort are divided as follows: 28 patients (41.8%) presented to hospitalization in phase 1; 34 (50.7%) in phase 2; 5 (7.46%) in phase 3. On the other hand, a classification on the severity of respiratory failure, we had: 36 patients (53.73%) presented as a mild case, requiring low-flow oxygen therapy; 25 patients (37.31%) required NMI; 8 patients (11.94%) returned to severe cases, requiring transfer to ICU (Figure 1).

DOI: 10.26717/BJSTR.2023.49.007737

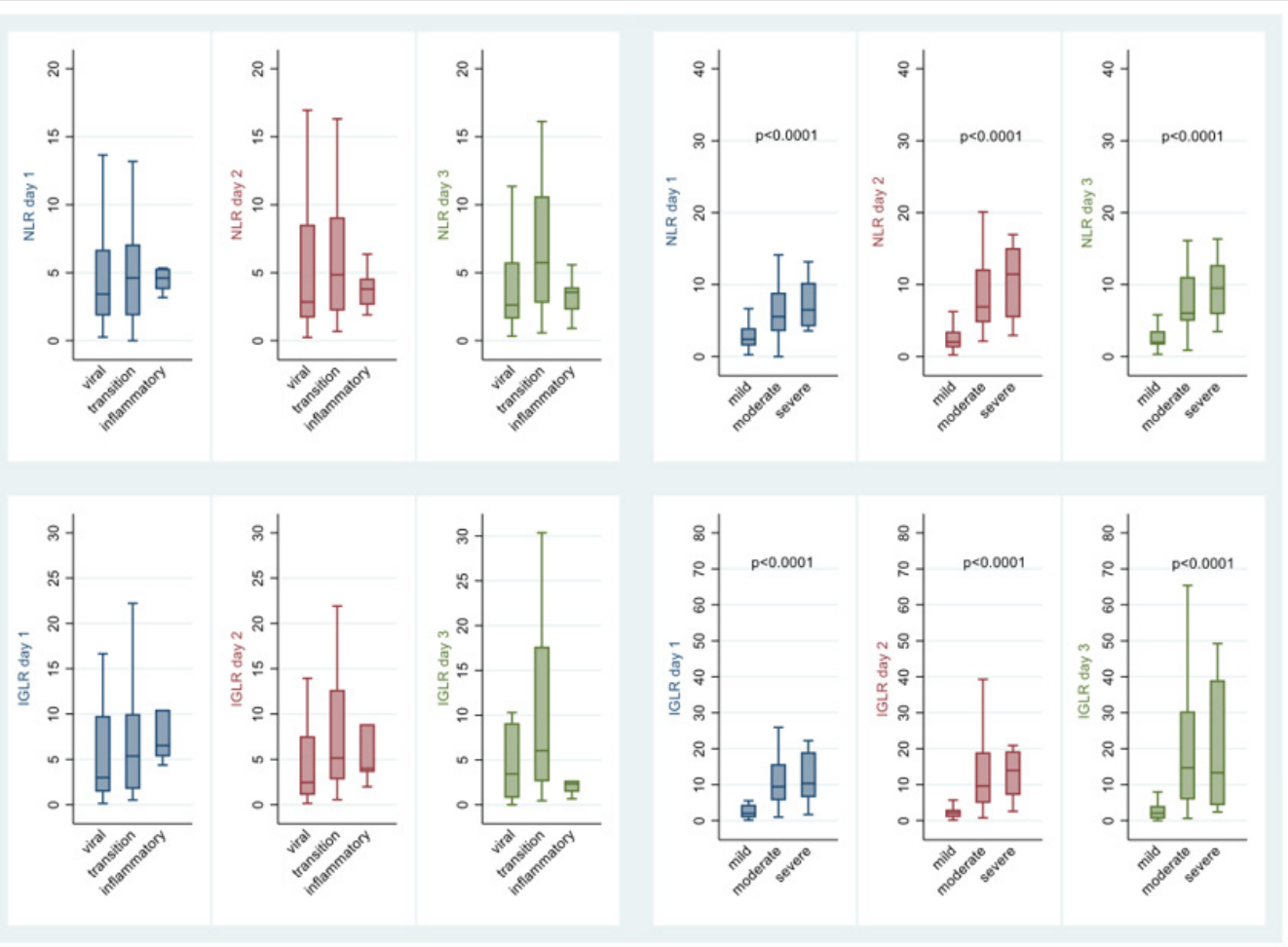

Figure 1: Trend of the NLR and IGLR parameter in the disease phases and in the severity classification.

WBC Findings

WCBs remain within the normal range during the first three days of hospitalization. As regards the comparison with the disease phase (viral -1-, transition -2-, inflammatory or 3) the following results emerged. There is a significant difference with the absolute number of white blood cells on day 1, in fact it is found between patients in the inflammatory phase 3 vs phase 1 (median phase 3: 9.1*10 ^ 3/ ul vs median phase 1: 5.1*10^3/ul, p 0.010). There is no difference between the white blood cell count on day 2 and 3. Moreover, there is also a difference between the neutrophil value and the disease phase on day 1, above all, this difference is present between the 3rd phase and that of the first (median phase 3: 6.6*10 ^ 3 / ul; median phase 1: 4*10 ^ 3 / ul, p=0.010). Mild lymphopenia was present during the three days in the studies. There is a correlation with the lymphocyte count and the disease phase on day 1. The lymphocytes values were statistically lower in phase 2 than in phase 3 (median phase 2: 0.9*10 ^ 3/ul; median phase 3 1.5*10^3/ul, p=0.024). There is no relationship of lymphocytes at day two, while there is a correlation on day three: again, the difference is present between the inflammatory and transitional phases (median phase 2: 0.9*10^3/ul; median phase 3 1.9*10^3/ul, p=0.022).

On the other hand, from this type of comparison no association emerge between the values of basophils, monocytes, eosinophils, immature granulocytes, re-lymph and the phases of the disease throughout the observation period. That are more links, maintained over time, when we correlate the results of the white blood cell series with the severity of the disease, calculated as the increased oxygen demand: mild, moderate and serious cases. There is a difference between neutrophils and disease severity. In particular, on 1st day, it is present among mild cases patients, who have lower neutrophils respect to moderate cases (p=0.006). While, on a 2nd and 3rd days the difference was present between severe cases with higher neutrophils versus mild cases (2nd day p=0.002, 3rd day p<0.001) and be-tween moderate and mild cases (2nd and 3rd days p<0.001). (Table 1). Lymphocytes were lower as gravity increases of disease and maintain this difference during the first three days. The difference was statistically significant especially between severe and mild cases (p=0.011, p= 0.002, p=0.003; 1st, 2nd and 3rd days respectively) and between moderate and mild cases (p<0.001 for 1st, 2nd and 3rd days respectively) (Table 1).

 DOI: 10.26717/BJSTR.2023.49.007737

Table 1: Neutrophils and lymphocytes on three days of the study.

Neutrophils(10^3/uL)			
	Mild Case	**Moderate Case**	**Severe Case**
1st day	median=3.3* (IQR 2.0-4,8)	median=5.1* (IQR 4.1-7.2)	median=4.5 (IQR 4.0-6.6)
	mean=3.7 (sd 2.2)	mean=5.9 (sd 3.9)	mean=5.6 (sd 2.5)
2nd day	median=3.1*° (IQR 1.9-3.6)	median=6.6* (IQR 3.8-8.6)	median=6.5° (IQR 5.7-7.3)
	mean=3.3 (sd 1.9)	mean=6.8 (sd 3.6)	mean=6.6 (sd 1.0)
3rd day	median=3.2*° (IQR 2.0-4,8)	median=6.4* (IQR 5.3-7.2)	median=7.5° (IQR 6.6-8.0)
	mean=3.6 (sd 2.3)	mean=6.5 (sd 2.8)	mean=7.2 (sd 1.6)
Lymphocytes(10^3/uL)			
	Mild case	Moderate Case	Severe Case
1st day	median=1.4*° (IQR 1.0-1.7)	median=0.8* (IQR 0.6-1.0)	median=0.8° (IQR 0.7-1.0)
	mean=1.5 (sd 1.0)	mean=0.9 (sd 0.4)	mean=0.8 (sd 0.3)
2nd day	median=1.4*° (IQR 1.1-1.8)	median=0.8* (IQR 0.7-1.2)	median=0.6° (IQR 0.5-1.0)
	mean=1.5 (sd 0.9)	mean=0.9 (sd 0.4)	mean=0.8 (sd 0.5)
3rd day	median=1.4*° (IQR 1.1-1.9)	median=0.8* (IQR 0.5-1.2)	median=0.08° 8(IQR 0.6-1.2)
	mean=1.6 (sd 1.0)	mean=1.0 (sd 2.2)	mean=0.9 (sd 0.3)

Note: *°p-value<α/3 for Bonferroni multiple testing correction.

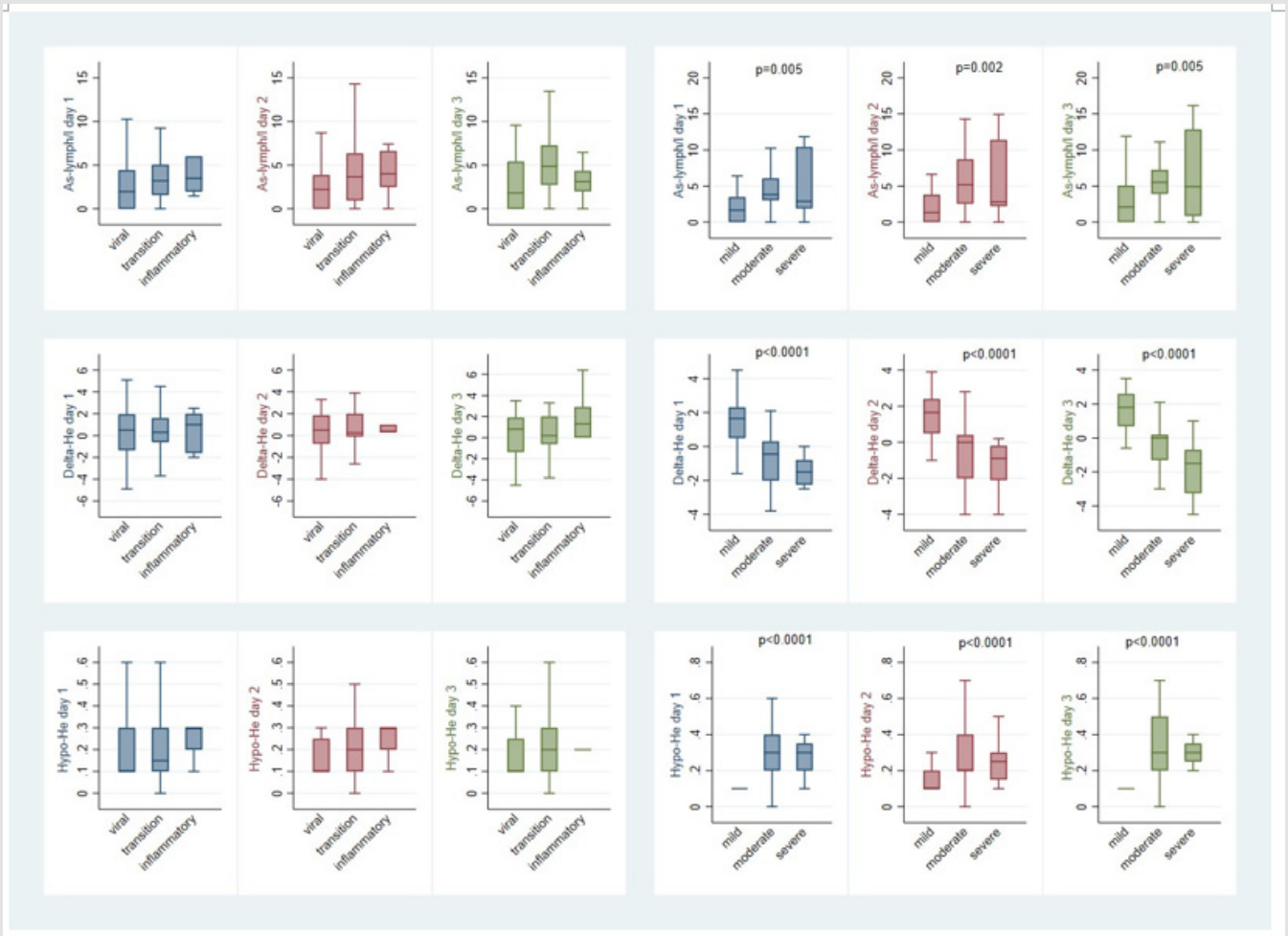

Figure 2: Trend of the AsLymph, Delta-He and Hypo-He in the disease phases and in the severity classification.

The relationship between neutrophils and lymphocytes (NRL) showed a progressive in-crease over time and in particular statistically higher levels between moderate and mild case ($p<0.001$ for 1st, 2nd and 3rd day respectively) and between case severe and moderate ($p=0.002$, $p<0.001$, $p<0.001$ on the 1st, 2nd and 3rd days respectively) (Figure 1). Immature granulocytes (GIs), normally absent in peripheral blood and the ratio immature granulocytes and the absolute number of lymphocytes (IGRL), increased in the three days of observation, and they were higher in severe and moderate cases, maintaining a significant difference with mild cases during the study period (Figure 1). The As-lymph /L ratio, that is the ratio between antibody-producing lymphocytes (As-lymph) and lymphocyte counts maintains a statistically significant difference in the first three days of hospitalization. In detail, it is always present higher levels in serious cases respect to mild case ($p=0.002$, $p=0.001$, $p=0.002$; 1st, 2nd and 3rd day respectively), and statistically lower values in mild cases (Figure 2).

RBC Findings

Reticulocyte value remained low during the study period. No relationship was found between red blood cell values and disease stage, while follow-ing results emerge when we compare the red blood cell data with the severity of the disease. The Delta-He and Hypo-He showed statistically significant differences in the three days of hospitalization. In detail between mild and moderate cases (for Hypo-He $p<0.001$; for Del-ta-He, $p<0.001$ for 1st, 2nd and 3rd respectively) and between severe and moderate (for Hypo-He $p=0.004$, $p=0.027$, $p<0.001$; for Delta-He, $p<0.001$ for 1st, 2nd and 3rd day respectively). In fact, Delta-He is lower with the increasing the gravity of disease, while Hy-po-He appears to increase (Figure 2).

Inflammatory Biomarkers Findings

No relationship was found between indexes of inflammations and disease stage, while following results emerge when we compare these data with the severity of the disease. The enzyme Lactate Dehydrogenase (LDH), in all three days of study, shows an increase over time, it was higher to mild cases respect to moderate cases ($p<0.001$, $p = 0.001$, $p = 0.004$ for 1st, 2nd and 3rd day respectively) and between case severe and mild ($p<0.001$ for 1st, 2nd and 3rd day respectively) (Table 2). The CRP have a significant difference only on the first day, between severe and mild cases ($p=0.001$) and between severe and moderate cases ($p=0.042$). On the contrary, PCT maintains this difference for all three days in particular between severe and mild cases ($p=0.001$; $p=0.001$; $p=0.012$ for 1st, 2nd and 3rd day respectively) (Table 2).

Table 2: LDH, CRP and PCT parameters on three days of the study.

LDH(U/1)			
	Mild Case	**Moderate Case**	**Severe Case**
1st day	median=224.0*° (IQR 194.5-278.5)	median=344.0* (IQR 266.04-416.0)	median=4.5 (IQR 353.5-788.0)
	mean=234.2 (sd 53.3)	mean=345.9 (sd 11.6.1)	mean=595.9 (sd 344.6)
2nd day	median=214.5*° (IQR 192.0-257.0)	median=325.5* (IQR 234.0-4.414.0)	median=444.0° (IQR 299.5-6.09.0)
	mean=223.3 (sd 78.6)	mean=341.0 (sd 131.0)	mean=450.6 (sd 173.9)
3rd day	median=220.0*° (IQR 194.0-0-262.0)	median=288.0*° (IQR 241.0-368.0)	median=551.0° (IQR 424.0-676.5)
	mean=234.3 (sd 70.0)	median=308.9 (sd 106.0)	median=579.1 (sd 213.5)
CRP (mg/L)			
	Mild cases	Moderate Cases	Severe Cases
1st day	median=40.2* (IQR 13.3-80.5)	median=62.8° (IQR 27.4-111.7)	median=111.2*° (IQR 91.2-194.8)
	mean=181.4 (sd 733.7)	mean=78.5 (sd 65.8)	mean=134.4 (sd 53.6)
PCT(ng/ml)			
1st day	median=0.1*° (IQR 0.0-0.1)	median=0.1* (IQR 0.0-0.2)	median=0.7° (IQR 0.1-1.2)
	mean=0.1 (sd 0.1)	mean=0.2 (sd 0.2)	mean=0.8 (sd 0.7)
2nd day	median=0.1* (IQR 0.0-0.1)	median=0.1* (IQR 0.0-0.1)	median=0.6* (IQR 0.1-2.1)
	mean=0.1 (sd 0.1)	mean=0.4 (sd 1.1)	mean=27.4 (sd 75.1)
3rd day	median=0.1* (IQR 0.0-0.1)	median=0.1 (IQR 0.0-0.1)	median=0.3* (IQR 0.1-1.1)
	mean=0.1 (sd 0.2)	mean=2.1 (sd 9.6)	mean=0.6 (sd 0.6)

Note: *°p-value<α/3 for Bonferroni multiple testing correction.

IL-6, a pivotal cytokine in self-sustaining inflammatory storm, remains higher during the first three days of hospitalization in severe cases, resulting in a statistically significant difference with the other groups during the observation period. Above all, this difference is present in all three levels of severity of the disease, for severe case vs mild case p=0.004, p<0.001, p<0.001, 1st, 2nd and 3rd day respectively; and between severe case vs moderate case (p=0.020, p=0.016, p=0.002; 1st, 2nd and 3rd respectively) (Figure 3). Ferritin is confirmed to be higher in severe patients. It remained higher in the three days observed, maintaining a difference between mild and moderate cases (p=0.002, p=0.001, p=0.031 for 1st, 2nd and 3rd day respectively) and between severe and mild case (p=0.002, p=0.002, p=0.001 for 1st, 2nd and 3rd day respectively) (Figure 3). D-Dimero finds no differences when compared with the stage of disease nor with the se-verity of the disease.

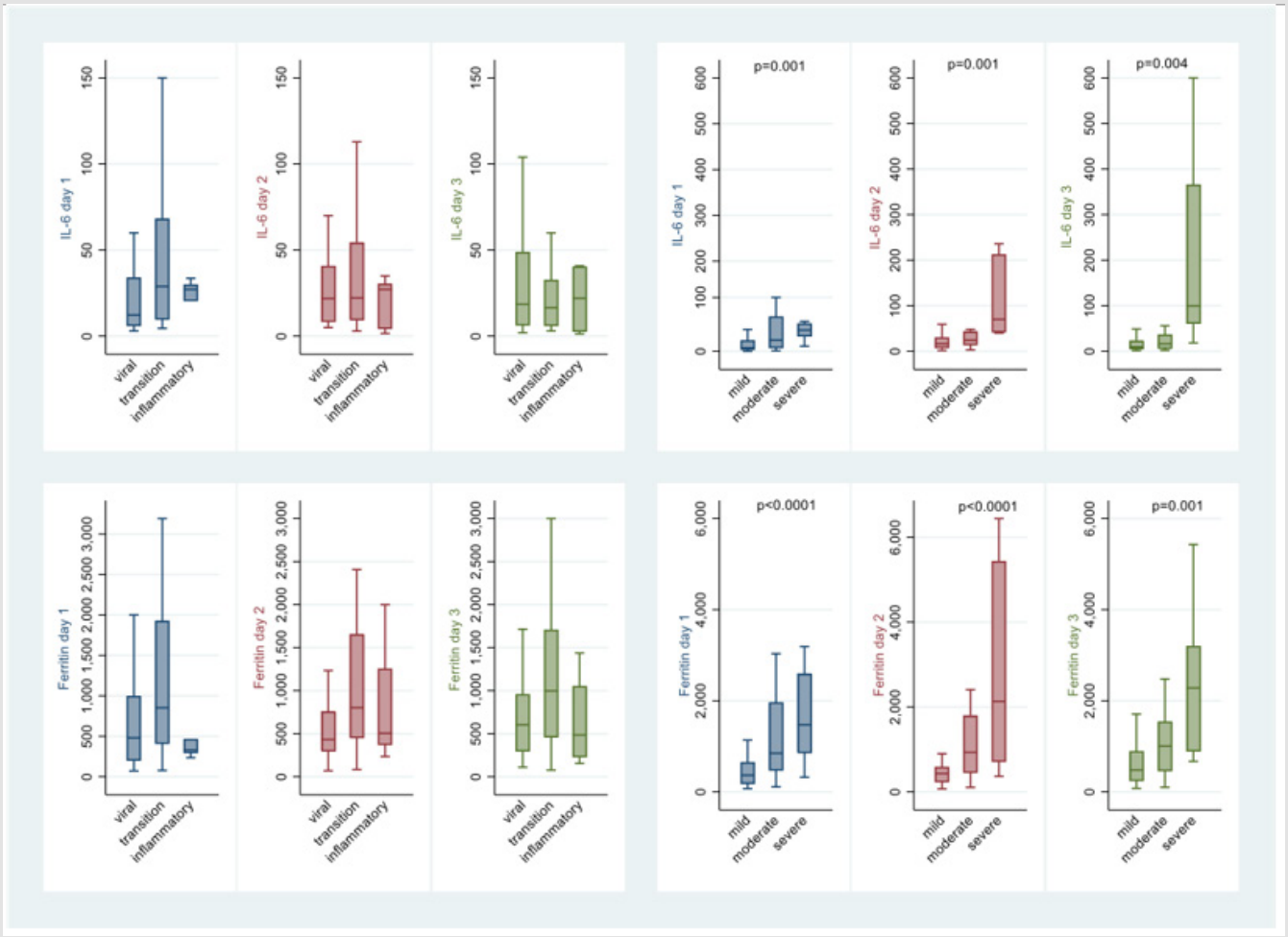

Figure 3: Trend of the IL-6 and ferritin in the disease phases and in the severity classification.

Discussion

The study showed, in a cohort of Italian patients diagnosed with COVID-19, that any WBC and RBC parameters can be useful to stratify the risk of evolution of the SARS-coV-2 infection, in the first days of hospidalitation. The aim of study was recognize the clinical phenotype of patients who should are expected to have moderate and severe disease, through hematochimical parameters, which are easy and quick to perform, and who may require different and more specific treatment protocols. In fact dosing of factors such as NLR, IGLR, As-lymph/L, Delta-He, Hypo-He, parameters extrapolated by haemocytometric examination, such as Il-6 and LDH could be useful for early identification of patients at high risk of severe/moderate respiratory failure. In a systematic review by Fu L., involving 43 studies and 3600 patients, the most common abnormalities detected in Covid-19 were decreased lymphocyte count (57.4%), an elevated CRP (68.6%) and an increase in LDH (51.6%) [14]. In our previous study we showed that a COVID-19 cohort admitted in emergency room, had a lymphopenia in 58.9% of cases, elevated values of CRP, a rise of D-dimer and of LDH. The neutrophil count was normal in 69.5% [15]. At the moment, there are no studies that analyze these blood parameters in the first three days of hospitalization respect to

the phases of disease and clinical score. We have shown that the count of white blood cells, lymphocytes, neutrophils, basophils, immature granulocytes, As-lymph, AST, platelets have a relationship with the disease phase, this is not present in all three days under study.

When we compare the data with the severity of the disease, based on different oxygen therapy supports, the relations appear more evident [16-19]. In the present study, it appears that the ratio of neutrophils to lymphocytes (NLR) is high in the most serious patients and that this ratio remains consistently high in the first three days of hospitalization. Consequently, we also find that neutrophils in absolute numbers are higher in severe patients and lymphocytes are lower. Hence, our findings are shown to be consistent with others from previous studies on the relationship between NLR and prognosis of many other infectious diseases [20-23]. Also the neutrophil (NEU) is an important component of the leukocyte population that activates and migrates where infection/inflammation is pre-sent. It releases large amounts of reactive oxygen species that damage cellular DNA and destroy viruses. Furthermore, NEU can be activated by inflammatory factors linked to the presence of viruses, such as interleukin-6 and interleukin-8, tumor necrosis factor alpha, granulocyte colony stimulating factor and interferon-gamma, produced by lymphocytes and endothelial cells [24-26]. On the other hand, systemic inflammation, triggered by SARS-CoV-2, significantly depresses cellular immunity, leading to a decrease in CD3 + T cells, CD4 + T cells, and CD8 + T cells. SARS-CoV-2 infected T cells can also cause cyto-pathic effects on T cells [27-29]. Therefore, NLR is an inexpensive marker that derives from routine peripheral blood tests and can be associated with the progression and prognosis of COVID-19.

Immature granulocytes (and granulocytes to lymphocytes ratio), represent metamyelocytes, myelocytes, promyelocytes. They are commonly present in our study population, especially in critically ill patients, unlike common population which does not have them in circulation. On the other hand, in peripheral blood immature granulocytes increase in response to infection, inflammation or other causes of bone marrow stimulation [30]. Severe insults such as sepsis, trauma and viral infections can induce emergency granulopoiesis, a hematopoietic response that rapidly increases the de novo production of neutrophils to meet the growing demands. This mechanism causes the presence of both immature and mature neutrophils populations in the peripheral blood, which can act as either immunosuppressive or proinflammatory [31,32].

Despite limited knowledge about the contribution of mature and immature neutrophils to the immune response and their distinct characteristics, clinical interest in these cells is growing due to their increasingly evident correlation with disease severity and/or response to treatment in many diseases, such as sepsis, severe flu and COVID-19 [6,33-36]. Furthermore, it has also been evaluated as a marker of acute respiratory distress syndrome, allowing to stratify the risk of evolution into ARDS [19, 37]. In fact, even in our study, the patients have an increase of NLR IGRL and As Lymphocytes in severe cases, with higher ratio than mild forms of disease, and this ratio increases over time. Many papers document an increase in activated lymphocytes in COVID-19 patients [38,39]. Our results agree with previously published studies reporting As-lymph as a parameter capable of predicting clinical severity in SARS-coV-2 patients [40,41]. During the course of the infection, moderate/severe cases probably show a significant decrease in total lymphocytes with an increase in those activated, capable of producing antibodies in response to the viral insult.

Taliani, et al., showed a correlation between IL-6 overexpression in patients with Covid-19 and the presence of hyperactivated plasmacytoid lymphocytes in peripheral blood [42]. In another study comparing patients with bacterial and viral infections, As-lymph was higher in the latter and even provided the same discriminatory power as procalcitonin between the two types of infections [43]. In the literature, erythropoietic changes have been reported during infection; these mostly show low levels of HGB [44,45]. The hemoglobinization of reticulocytes, as indicated by negative levels of Delta-He, is however significantly impaired, particularly in the most se-vere cases, probably due to ongoing inflammation, as showed in our study [46]. Ferritin is a protein that stores iron; its serum level reflects the normal level of iron and helps in the diagnosis of iron deficiency anemia. The circulating ferritin level increases during viral infections and may be a marker of viral replication [47,48]. Increased ferritin levels due to cytokine storm and secondary haemophagocytic lymphohistiocytosis (sHLH) have also been reported in severe patients with COVID-19, as confirmed by our study [49,50].

In most diseases, the circulating CRP value reflects ongoing inflammation and/or tissue damage much more accurately than other laboratory parameters of acute phase response. The CRP concentration should therefore be a very useful non-specific biochemical marker of inflammation, the measurement of which contributes significantly to monitoring the response to treatment of inflammation and infection [51]. As can be seen in our study, this probably also happens in patients with COVID-19, where the response of the CRP is not maintained for the entire time of observation. During the inflammatory storm in COVID-19, many inflammatory cytokines are rapidly produced, including IL-6, TNF-α, IL-1β, IL-12, and IFN-γ, which stimulate hepatocytes, Kupffer cells, and macrophages to secrete ferritin. In particular, ferritin is not only the re-sult of excessive inflammation, but also plays a pathogenic role in the inflammatory process through its binding to T-cell immunoglobulin and mucin domain 2 (TIM-2) by promoting expression of multiple inflammatory promoters [52]. Furthermore, some studies have shown that the ferritin H chain activates macrophages to secrete inflammatory cytokines [53]. IL-6 is a multifunctional cytokine involved in the transmission of cellular signals and in the regulation of immune cells. This factor has a strong proinflammatory effect and it plays an important role in tumors and hematological diseases. Meta-analyzes available in the literature indicate that increased IL-6 levels are significantly associated with

adverse clinical outcomes, including ICU admission, ARDS and death [54]. Indeed, dysregulated immune response to infectious pathogens represents an important contribution to morbidity and mortality. Our data also show an increase in IL-6 and ferritin levels in critically ill patients, noting that the cytokine storm is a crucial event for COVID-19 progression.

LDH is an intracellular enzyme present in the cells of almost all organ systems, which catalyzes the interconversion of pyruvate and lactate, with concomitant interconversion of NADH and NAD + [55]. Although, traditionally LDH were used as a marker of heart damage, abnormal values can result from injury to multiple organs and decreased oxygenation with upregulation of the glycolytic pathway. Because LDH is present in lung tissue (isozyme 3), it can be expected that patients with severe. COVID-19 releases larger quantities of LDH into circulation, as can be seen from our data. Therefore, in the presence of a severe form of interstitial pneumonia, which often progresses to acute respiratory distress syndrome, this value can be used as a hallmark of worsening of the disease. The present study has some limitations, such as the cross-sectional nature of the study and the fact that it was monocentric, the low sample size and the lack of evaluation of other markers with in vitro and in vivo effects that may provide further insight into the mechanisms linking these alterated haematochimical parameters with the disease evolutions. Finally, additional mechanistic studies are needed to further elucidate the roles of these markers and their signalling in populations with COVID19.

Conclusion

Early identification and proper treatment of COVID-19 patients at high risk for acute respiratory failure is critical to avoiding ARDS and organ damage. Based on our results, we believe that classic inflammation markers such as CRP are not sufficient. Instead, the dos-ing of factors such as NLR, IGLR, As-lymph/L, Delta-He, Hypo-He, IL-6, LDH, ferritin could be useful for the early identification of patients at high risk of moderate/severe acute respiratory failure. These patients, all having a profile of greater complexity related to hyper inflammation, could benefit from more careful observation and timely correct treatment. Timely and specific treatments such as the use of antivirals or monoAb can improve prognosis and the risk of disease progression.

Author Contributions

For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, MP; methodology, ML; formal analysis, PB and MDN; MP and KF writing-original draft preparation, JV and CU; writing-review and editing. All authors have read and agreed to the published version of the manuscript."

Funding

This research received no external funding.

Institutional Review Board Statement

"The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of University G. d'Annunzio" Chieti.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

Not applicable.

Acknowledgments

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Guan W, Ni Z, Hu Y, Liang W, Ou C, et al. (2020) Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med 382: 1708-1720.
2. Sun Q, Qiu H, Huang M, Yang Y (2020) Lower mortality of COVID-19 by early recognition and intervention: experience from Jiangsu Province. Ann Intensive Care p. 10.
3. Siddiqi HK, Mehra MR (2020) COVID-19 illness in native and immunosuppressed states: A clinical–therapeutic staging pro-posal. The Journal of Heart and Lung Transplantation 39: 405-407.
4. Ucciferri C, Vecchiet J, Falasca K (2020) Role of monoclonal antibody drugs in the treatment of COVID-19. World Journal of Clinical Cases 8: 4280-4285.
5. Romp KG, Peters WP, Hoffman M (1994) Reticulated platelet counts in patients undergoing autologous bone marrow trans-plantation: an aid in assessing marrow recovery. Am J Hematol 46: 319-324.
6. Buttarello M, Temporin V, Ceravolo R, Farina G, Bulian P (2004) The new reticulocyte parameter (RET-Y) of the Sysmex XE 2100: its use in the diagnosis and monitoring of posttreatment sideropenic anemia. Am J Clin Pathol 121: 489-495.
7. Chabot Richards DS, George TI (2015) White blood cell counts: reference methodology. Clin Lab Med 35: 11-24.
8. Toori KU, Qureshi MA, Chaudhry A (2021) Lymphopenia: A useful predictor of COVID-19 disease severity and mortality. Pak J Med Sci 37: 1-5.
9. Xinyi Zhao, Lijin Zhou, Yan Kou, Junjie Kou (2022) Activated neutrophils in the initiation and progression of COVID-19: hyperinflammation and immunothrombosis in COVID-19 -PubMed 14(3): 1454-1468.
10. Fu L, Wang B, Yuan T, Chen X, Ao Y, et al. (2020) Clinical characteristics of coronavirus disease 2019 (COVID-19) in China: A systematic review and meta-analysis. J Infect 80: 656-665.
11. Ucciferri C, Caiazzo L, Di Nicola M, Borrelli P, Pontolillo M, et al. (2021) Parameters associated with diagnosis of COVID-19 in emergency department. Immun Inflamm Dis 9(3): 851-861.
12. Ucciferri C, Barone M, Vecchiet J, Falasca K (2020) Pidotimod in paucisymptomatic SARS-CoV2 infected patients. Mediterrane-an Journal of Hematology and Infectious Diseases 12(1): e2020048.
13. Katia F, Myriam DP, Ucciferri C, Auricchio A, di Nicola M, et al. (2021) Ef-

ficacy of canakinumab in mild or se-vere COVID-19 pneumonia. Immun Inflamm Dis 9(2): 399-405.

14. Ucciferri C, Auricchio A, di Nicola M, Potere N, Abbate A, et al. (2020) Canakinumab in a subgroup of patients with COVID-19. The Lancet Rheumatology 2(8): e457-e458.
15. D'ardes D, Pontolillo M, Esposito L, Masciarelli M, Boccatonda A, et al. (2020) Duration of covid-19: Data from an ital-ian cohort and potential role for steroids. Microorganisms 8(9): 1327.
16. Huang Z, Fu Z, Huang W, Huang K (2020) Prognostic value of neutrophil-to-lymphocyte ratio in sepsis: A meta-analysis. Am J Emerg Med 38: 641-647.
17. Liu Y, Du X, Chen J, Jin Y, Peng L, et al. (2020) Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. J Infect 81: e6-e12.
18. Bedel C, Korkut M (2021) Neutrophil-to-Lymphocyte Ratio and Covid-19. Shock 56: 874.
19. Şentürk M, Azgın İ, Övet G, Alataş N, Ağırgöl B, et al. (2016) The role of the mean platelet volume and neutrophil-to-lymphocyte ratio in peritonsillar abscesses. Braz J Otorhinolaryngol 82: 662-667.
20. Blaser MJ, Chyou PH, Nomura A (1995) Age at Establishment of Helicobacter pylori Infection and Gastric Carcinoma, Gastric Ulcer, and Duodenal Ulcer Risk. Cancer Res 55: 562-565.
21. Scholl SM, Pallud C, Beuvon F, Hacene K, Stanley ER, et al. (1994) Anti-Colony-Stimulating Factor-1 Anti-body Staining in Primary Breast Adenocarcinomas Correlates with Marked Inflammatory Cell Infiltrates and Prognosis. JNCI: Journal of the National Cancer Institute 86: 120-126.
22. Shacter E, Weitzman SA (2002) Chronic inflammation and cancer. Oncology (Williston Park) 16(2): 217-226.
23. Azkur AK, Akdis M, Azkur D, Sokolowska M, van de Veen W, et al. (2020) Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy 75: 1564-1581.
24. Van Der Poll T, Van De Veerdonk FL, Scicluna BP, Netea MG (2017) The immunopathology of sepsis and potential therapeutic targets. Nat Rev Immunol 17: 407-420.
25. Zhang X, Tan Y, Ling Y, Lu G, Liu F, et al. (2020) Viral and host factors related to the clinical outcome of COVID-19. Na-ture 583: 437-440.
26. Myari A, Papapetrou E, Tsaousi C (2021) Diagnostic value of white blood cell parameters for COVID-19: Is there a role for HFLC and IG? Int J Lab Hematol 44(1): 104-111.
27. Scapini P, Marini O, Tecchio C, Cassatella MA (2016) Human neutrophils in the saga of cellular heterogeneity: insights and open questions. Immunol Rev 273: 48-60.
28. Manz MG, Boettcher S (2014) Emergency granulopoiesis. Nat Rev Immunol 14: 302-314.
29. Dylan Kotliar, Aaron E Lin, James Logue, Travis K Hughes, Nadine M Khoury, et al. (2020) Single-Cell Profiling of Ebola Virus Disease In Vivo Reveals Viral and Host Dynamics | Elsevier Enhanced Reader 183(5): 1383-1401.
30. Reusch N, De Domenico E, Bonaguro L, Schulte Schrepping J, Baßler K, et al. (2021) Neutrophils in COVID-19. 12: 652470.
31. Schulte Schrepping J, Reusch N, Paclik D, Baßler K, Schlickeiser S, et al. (2020) Severe COVID-19 Is Marked by a Dysregulated Myeloid Cell Compartment. Cell 182: 1419-1440.e23.
32. De Santo C, Salio M, Masri SH, Lee LYH, Dong T, et al. (2008) Invariant NKT cells reduce the immunosuppressive activity of influenza A virus-induced myeloid-derived suppressor cells in mice and humans. J Clin Invest 118: 4036-4048.
33. Ucciferri C, Vecchiet J, Falasca K (2020) Role of monoclonal antibody drugs in the treatment of COVID-19. World J Clin Cases 8: 4280-4285.
34. Huang Y, Xiao J, Cai T, Yang L, Shi F, et al. (2019) Immature granulocytes: A novel biomarker of acute respiratory dis-tress syndrome in patients with acute pancreatitis. J Crit Care 50: 303-308.
35. Chong VCL, Lim KGE, Fan BE, Chan SSW, Ong KH, et al. (2020) Reactive lymphocytes in patients with COVID-19. Br J Haematol 189: 844.
36. Gérard D, Henry S, Thomas B (2020) SARS-CoV-2: a new aetiology for atypical lymphocytes. Br J Haematol 189: 845.
37. Linssen J, Ermens A, Berrevoets M, Seghezzi M, Previtali G, et al. (2020) A novel haemocytometric COVID-19 prognostic score developed and validated in an observational multicentre European hospital-based study. Elife 9: 1-37.
38. Yip CYC, Yap ES, De Mel S, Teo WZY, Lee CT, et al. (2020) Temporal changes in immune blood cell parameters in COVID-19 infection and recovery from severe infection. Br J Haematol 190: 33-36.
39. Taliani G, Follini E, Guglielmetti L, Bernuzzi P, Faggi A, et al. (2021) Case Report: B Lymphocyte Disorders Under COVID-19 Inflammatory Pressure. Front Oncol p. 10.
40. Henriot I, Launay E, Boubaya M, Cremet L, Illiaquer M, et al. (2017) New parameters on the hematology analyzer XN-10 (SysmexTM) allow to distinguish childhood bacterial and viral infections. Int J Lab Hematol 39: 14-20.
41. Taneri PE, Gómez Ochoa SA, Llanaj E, Raguindin PF, Rojas LZ, et al. (2020) Anemia and iron metabolism in COVID-19: a systematic review and meta-analysis. Eur J Epidemiol 35: 763-773.
42. Yağcı S, Serin E, Acicbe Ö, Zeren Mİ, Odabaşı MS (2021) The relationship between serum erythropoietin, hepcidin, and hapto-globin levels with disease severity and other biochemical values in patients with COVID-19. Int J Lab Hematol 43: 142-151.
43. Weimann A, Cremer M, Hernáiz Driever P, Zimmermann M (2016) Delta-He, Ret-He and a New Diagnostic Plot for Differen-tial Diagnosis and Therapy Monitoring of Patients Suffering from Various Disease-Specific Types of Anemia. Clin Lab 62: 667-677.
44. Li Y, Hu Y, Yu J, Ma T (2020) Retrospective analysis of laboratory testing in 54 patients with severe- or critical-type 2019 novel coronavirus pneumonia. Lab Invest 100: 794-800.
45. Baraboutis IG, Gargalianos P, Aggelonidou E, Adraktas A (2020) Initial Real-Life Experience from a Designated COVID-19 Centre in Athens, Greece: a Proposed Therapeutic Algorithm. SN Compr Clin Med 2: 689-693.
46. Feld J, Tremblay D, Thibaud S, Kessler A, Naymagon L (2020) Ferritin levels in patients with COVID-19: A poor predictor of mortality and hemophagocytic lymphohistiocytosis. Int J Lab Hematol 42: 773-779.
47. Cheng L, Li H, Li L, Liu C, Yan S, et al. (2020) Ferritin in the coronavirus disease 2019 (COVID-19): A systematic re-view and meta-analysis. J Clin Lab Anal p. 34.
48. Pepys MB, Hirschfield GM (2003) C-reactive protein: a critical update. Journal of Clinical Investigation 111: 1805.
49. Kernan KF, Carcillo JA (2017) Hyperferritinemia and inflammation. Int Immunol 29: 401-409.
50. Torti FM, Torti S V (2002) Regulation of ferritin genes and protein. Blood 99: 3505-3516.
51. Coomes EA, Haghbayan H (2020) Interleukin-6 in Covid-19: A systematic review and meta-analysis. Rev Med Virol 30: 1-9.
52. Hsu PP, Sabatini DM (2008) Cancer cell metabolism: Warburg and beyond. Cell 134: 703-707.

53. Henry BM, Aggarwal G, Wong J, Benoit S, Vikse J, et al. (2020) Lactate dehydrogenase levels predict coronavirus disease 2019 (COVID-19) severity and mortality: A pooled analysis. Am J Emerg Med 38: 1722.

54. Poggiali E, Zaino D, Immovilli P, Rovero L, Losi G, et al. (2020) Lactate dehydrogenase and C-reactive protein as predictors of respiratory failure in CoVID-19 patients. Clin Chim Acta 509: 135-138.

55. Bartziokas K, Kostikas K (2021) Lactate dehydrogenase, COVID-19 and mortality. Med Clin (Barc) 156: 37.

ISSN: 2574-1241

DOI: 10.26717/BJSTR.2023.49.007737

Katia Falasca. Biomed J Sci & Tech Res

Submission Link: https://biomedres.us/submit-manuscript.php