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RELATIONSHIP BETWEEN PRE-EXISTING CONDITIONS IN COVID-19 PATIENTS AND INFLAMMATION

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ABSTRACT

COVID-19 is characterized by a strong inflammatory response leading to specific changes in the immune system cell counts. In this study, the following biomarkers from the laboratory data of COVID-19 patients have been analyzed: white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosinophils, basophils, C-reactive protein, and glycemia. Various comparisons, correlations, and ratios between these inflammatory biomarkers have been performed to assess the link between pre-existing conditions and the various blood markers, as well as the disease severity. WBC, neutrophils, C-reactive protein, and glycemia had high levels in more than 60-70% of the patients, although diabetic patients had even higher glycemia levels ($p<0.05$). A high correlation was found between WBC and neutrophils which dominate the WBC count, and a more moderate one between lymphocytes and monocytes. Lymphocyte, monocyte, and basophil counts were lower in patients with comorbidities in general, while lymphocytes and monocytes were also lower in the subset of patients with diabetes in particular ($p<0.05$). The means and medians of all the aforementioned hematological parameters have been calculated for each subset of pre-existing conditions.

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Introduction

Covid-19 – General Aspects

Coronaviruses are members of the subfamily named orthocoronavirinae containing four genera: alphacoronavirus, beta coronavirus, gamma coronavirus and delta coronavirus. Alpha and beta coronaviruses infect only mammals and cause respiratory illness in humans [1]. The first coronaviruses were discovered in the 1960s, while the severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) was the first human coronavirus pathogen detected in 2002. Since then, seven human coronaviruses have been found [2]. Covid-19 belongs to the beta coronaviruses [3]. It is a single-stranded, nonsegmented positive polarity RNA genome virus. Its virion has four major structural proteins: nucleocapsid protein (N), transmembrane protein (M), an envelope protein (E), and spike protein (S), the last three forming the virus envelope [3]. The most abundant protein is M, being responsible for the virus shape. Proteins S and M are transmembrane proteins involved in virus assembly during replication. The N protein is associated with the virus RNA inside the envelope (CoV replication cycle) [4]. S proteins are responsible for the crown-like appearance of the virus and they also are the most immunogenic part because they will bind with angiotensin-converting enzyme 2 (ACE2) receptors to enter the host cell [4, 5].

COVID19 was detected for the first time in Wuhan (China) in December 2019, then started spreading rapidly across the world, being able to spread by human-to-human transmission. COVID19 was found to have > 95 % homology with the bat coronavirus and > 70 % similarity with the SARS-CoV [6]. The World Health Organization declared COVID19 to be a pandemic on the 11th of March 2020. Some of the patients were asymptomatic, and some had flu-like symptoms in the beginning, but later on, the virus became very lethal leading to a significant threat to public health [7].

COVID-19 incubation time according to clinical data is from 2 to 14 days. The clinical characteristics include severe acute respiratory syndrome, hyperinflammatory response, vascular damage, microangiopathy, angiogenesis, and widespread

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thrombosis [8]. The four stages involved in severe COVID-19 infection are upper respiratory tract infection, dyspnea, pneumonia, cytokine storm-producing hyperinflammatory state, and death or recovery. Most commonly, symptoms such as headache, loss of smell, nasal obstruction, cough, asthenia, myalgia, rhinorrhea, gustatory dysfunction, sore throat, and fever were noticed in patients with mild or moderate COVID-19 infection according to an observational study with 1420 patients [9].

Covid-19 and Inflammation

COVID-19 is a viral infectious disease that results in the activation of inflammation as an immune response. Immunity acts through cells and proteins to defeat the infection [8]. The major organs of the immune system are the thymus, liver, bone marrow, tonsils, lymph nodes, spleen, and blood [8]. The cells of the immune system include Lymphocytes (T-lymphocytes, B-lymphocytes, Natural Killer cells), Phagocytic cells (Monocytes, Macrophages), Granulocytic cells (Neutrophils, Basophils, Eosinophils), and Dendritic cells [8]. The immune system is divided into two categories: adaptive immune system (T and B lymphocytes) and innate immune system (granulocytes, monocytes, macrophages, NK cells, dendritic cells, lymphoid cells, and mast cells) [10].

T-Lymphocytes are divided into 3 categories: T helper cells (Th), T cytotoxic cells (Tc), and T suppressor cells (Ts). They react when the antigen binds to the T-cell membrane proteins known as Major Histocompatibility Complex (MHC I and MHC II) [11]. T helper cells (Th) present MHC II on the surface which will recognize and interact with the antigen; subsequently the complex will start the cytokines secretion [11]. Natural Killer cells (NK) produce lytic granules (containing perforin and granulysin) and also cytokines such as interferon [IFN]-γ, tumor necrosis factor (TNF)-α, interleukin [IL]-10. Most of the NK cell's function is to be cytotoxic [12]. IFNγ attracts the macrophages for phagocytosis and lysis, while TNFα accelerates the direct killing. NK cells provide a rapid response to virus infections [11].

Monocytes circulate in the bloodstream and migrate into the tissues where they will differentiate into specific tissue macrophages [11]. Macrophages, which are normally in the resting phase, will be activated by cytokines secreted by the activated Th cells and will eliminate potential pathogens by phagocytosis [11]. Granulocytes contain four different cell types: neutrophils, basophils, eosinophils, and mast cells, each having a different morphology. They all secrete cytokines, and they assist in the modulation of the adaptive immune response [6, 11].

Cytokines are small molecules, glycoproteins that play a significant role as modulators of inflammation [13]. Cytokines include interleukins (IL), chemokines, interferons, and tumor necrosis factors (TNF) [13]. Cytokine activation is a normal physiological phenomenon that occurs always in the stressed or infected cell, through receptor-ligand interactions, activating a large number of white blood cells. This starts first as a locally post-primary infection and then spreads throughout the body via systemic circulation. It triggers the classical signs of inflammation: calor (heat), dolor (pain), rubor (redness), tumor (swelling or edema), and finally loss of function [14]. COVID-19 causes uncontrolled and sudden cytokine release which in large quantities can cause multisystem organ failure and finally death.

At the beginning stage of the disease, the hematological tests showed an unchanged or decreased number of WBC and decreased lymphocytes (lymphopenia) [15]. Other pathologic features include high serum levels of ferritin and D-dimers, the tendency for monocytosis (rather than lymphocytosis), and low Natural Killer and cytotoxic T cell count [5]. The pathogen recognition by the immune system determines the appearance of Natural Killer cells and cytotoxic T-cells, causing large production of pro-inflammatory cytokines and chemokines (IL-1, IL-6, TNF- α, interferons) [5]. More cytokine-producing cells (macrophages, neutrophils, T-cells) increase in number, leading to destructive effects on endothelial tissue and damage to the vascular barrier, capillaries, and lung alveoli, multiorgan failure, and finally death [16].

C-reactive protein (CRP) is a pentameric acute phase protein that binds to foreign pathogens mediating innate immunity. Its levels can grow up to 10,000-50,000 times from normal values, depending on the infectious agents/aggressive stimuli (infection, inflammation, trauma) [17-19]. It is a key inflammation biomarker with clinical importance which was assessed in a wide range of pathologies [17-19].

A wide range of inflammatory markers, as well as the virus's structural proteins, have been used as useful tools for the diagnosis and monitoring of COVID-19, which is crucial for pandemic management. Various detection methods have been developed for pandemic surveillance: nucleic acid-based detection, serological assays, biosensors (as platforms for point-of-care devices), nanobodies assays, and radiology [20-23].

Covid-19 and Comorbidities

Even if COVID-19 infection can be contracted by anyone, the infection is more severe in people over 60 years old with other underlying diseases. For example, in the case of diabetes, many studies indicate that diabetic persons are more susceptible to the infection (the immune profile is changed, and ACE2 receptors are also found in the pancreas, increasing levels of furin) [24-26]. Concerning obesity, being overweight impairs the oxygen saturation in the blood (pulmonary ventilation is compressed at the base of the lungs) which will cause inflammation by secretion of abnormal amounts of cytokines, adipokines, and interferons [26]. Studies have shown that 47.6% of COVID-19 patients were obese, of which between 75%-85 % required mechanical ventilation [26, 27].

This study explores the link between pre-existing medical conditions (diabetes and obesity in particular and any comorbidity in general) and blood parameters (inflammatory parameters including white blood cells, neutrophils, lymphocytes, eosinophils, basophils, and C-reactive protein, as well as glycemia) at the time of admission. Some inflammatory biomarkers such as white

blood cells, neutrophils, and C-reactive protein, as well as glycemia, had increased levels in the majority of COVID-19 patients. Monocytes, lymphocytes, and basophils were lower in patients with comorbidities in general, while monocytes and lymphocytes were found to also be lower in patients with diabetes in particular ($p<0.05$). White blood cell count was dominated by neutrophils, which leads to a strong correlation between the two, while lymphocytes and monocytes had the second largest correlation. The correlations between all measured blood parameters, as well as the averages and medians for these parameters as a function of pre-existing conditions, have been also calculated.

Materials and Methods

The data from 52 patients who were admitted to the County Clinical Emergency Hospital of Oradea, Romania, between 01.11.2020-31.12.2020 has been collected from their specific observation paper (FO). The biochemical results of the blood analyses were obtained at the time of hospital admission by using the Architect c4000 (Abbott, USA). A positive PCR test for COVID-19 diagnosis had been used as inclusion criteria. Three groups of pre-existing conditions have been analyzed: diabetes mellitus, obesity, and general comorbidities. The graphics have been obtained by using Microsoft Excel and Origin software. Statistical results were evaluated using the IBM SPSS statistical processor for Windows, version 28.0.1.1.
The data about the patients with COVID-19 was collected through online access from the hospital (permission nr. 10538/04.04.2022). Permission from the Ethical Committee of the Faculty of Medicine and Pharmacy, University of Oradea, was also obtained (CEFMF/08/30.05.2022).

Results and Discussion

The measured number of the cells involved in the immune response generated by inflammation has been analyzed and plotted in the graphics below, classifying the values into 3 groups: normal, under, and over normal physiological values **(Figure 1a)**. The cells which were studied are the following: white blood cells (WBC, normal values are between 4,00 - 10,00 10x9/L), neutrophils (normal values are between 2.00 - 7,00 10x9/L), lymphocytes (normal values are between 0.80 - 4,00 10x9/L), monocytes (normal values are between 0.12 – 1.20 10x9/L), eosinophils (normal values are between 0.02 – 0.50 10x9/L) and basophils (normal values are between 0.00 – 0.10 10x9/L). Another biochemical parameter specific for inflammation, C-reactive protein (CRP, normal value is less than 10 mg/L), was also assessed (data available for only 21 patients) **(Figure 1c)**. Throughout the remaining of this paper, the units for immune cell counts (when not specified) are understood to be 10^9/L, for CRP mg/L, and for glycemia mg/dL.
The presence of pre-existing conditions was also evaluated: 14 patients presented diabetes mellitus, 23 patients obesity, and 8 patients had no comorbidities.

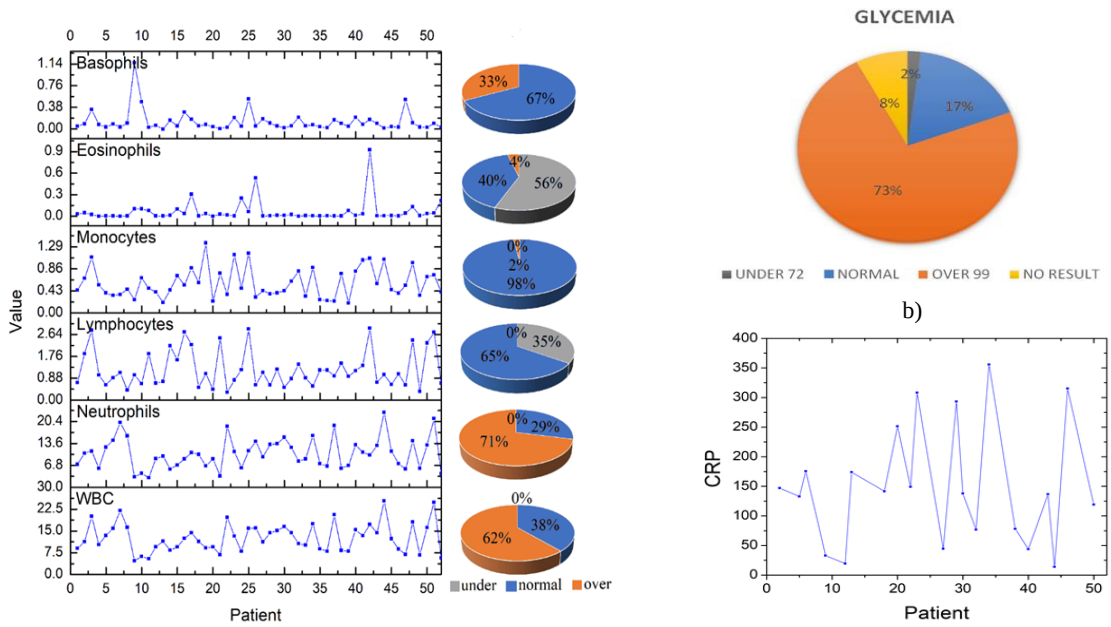


Figure 1. a) Immune cell counts and distribution of values that are normal, under and over normal physiological values for patients with COVID-19; b) Distribution of glycemia for patients with COVID-19; c) CRP values for patients with COVID-19

White Blood cells had high values in over 62% of the patients. WBC count will always be high in Covid-19 infection regardless of whether the patients have diabetes mellitus, obesity, hyperglycemia, or no comorbidity. Neutrophil levels were high in over 71% of the patients. Lymphocytes were normal in over 65% of cases. Monocyte values were normal in 98% of the patients.

Eosinophil values were high only in 4% of the patients and basophil values had normal values in over 67% of the patients' cases.

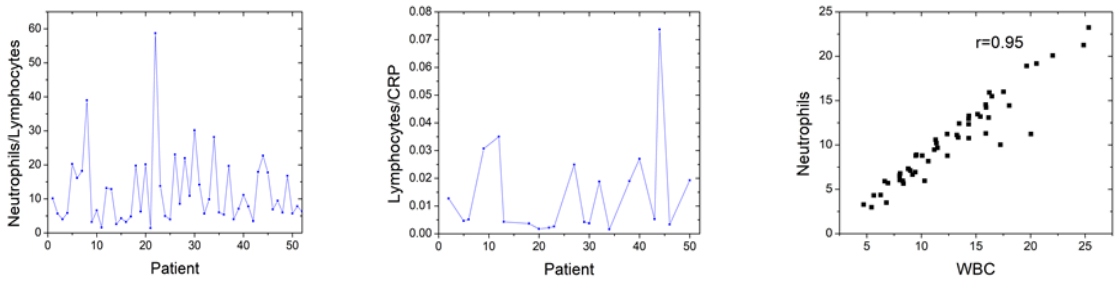
All the patients presented CRP values that were more elevated than normal, 14 patients of 21 had CRP values over 100 mg/L. CRP is usually always high in different inflammations, therefore we cannot conclude that CRP was high only due to Covid-19 inflammation or also due to pre-existing conditions.

The same tendency of increased levels for CRP and neutrophils was also reported by other authors [28-30], while the decreased number of eosinophils was pointed out in other studies for COVID-19 patients [29, 31, 32]. An influx of neutrophils and monocytes into the nasopharyngeal mucosa, and of proinflammatory macrophages into the lungs was shown in patients with COVID-19. Increased levels of eosinophils and reduced numbers of basophils were also reported [10]. Neutrophilia (an increase of neutrophils) and lymphopenia (decrease of lymphocytes) were introduced as a hallmark of disease severity, leading to an elevated neutrophil/lymphocyte ratio (for severe cases, the median ratio was 5.5 and the interquartile range was 3.3-10) [10, 33]. Another ratio that can predict the clinical severity of COVID-19 patients is a low lymphocyte/CRP ratio (for severe cases, the ratio of the medians was 14x10^-3 in a large study with a total of 452 patients) [10, 34].

Glycemia (normal values are between 72-99 mg/dl) was also taken into consideration and the distribution of its values is presented in **Figure 1b**. The glycemia values were significantly elevated in Covid-19 infected patients (with some values of more than 400 mg/dl) even though only 25% of the patients had been previously diagnosed with diabetes mellitus. Only around 20% of the patients were normoglycemic. Persons with no previous underlying disease represented 16% of the total number of patients. 45% of the patients were obese, of which 31% were hyperglycaemic and 23% had also diabetes mellitus.

The neutrophil/lymphocyte ratio and lymphocyte/CRP ratio were also assessed **(Figures 2a and 2b)** indicating that most of the patients presented a moderate or severe status with an enhanced systemic inflammatory process.

A strong correlation has been found between WBC and neutrophil count (r=0.95) **(Figure 2c)** mostly explained by the fact that in this study the WBC count is dominated by the neutrophil count.



a) b) c)

Figure 2. a) Neutrophil/lymphocyte ratio (median ratio 8.2, interquartile range 5.5-17.9); b) Lymphocyte/CRP ratio for patients with COVID-19 (the ratio of the corresponding medians is 7.4x10^-3, **(Table 2)**); c) Correlation between WBC and neutrophils count (the Pearson correlation coefficient is denoted by r

The correlation between any other pairs of parameters is less pronounced, and the next largest correlation is between monocytes and lymphocytes (r=0.505) **(Table 1)**. The CRP data was only available for 21 patients, while the rest of the data was for 52 patients.

Table 1. Pearson correlation coefficients (r) between measured markers.

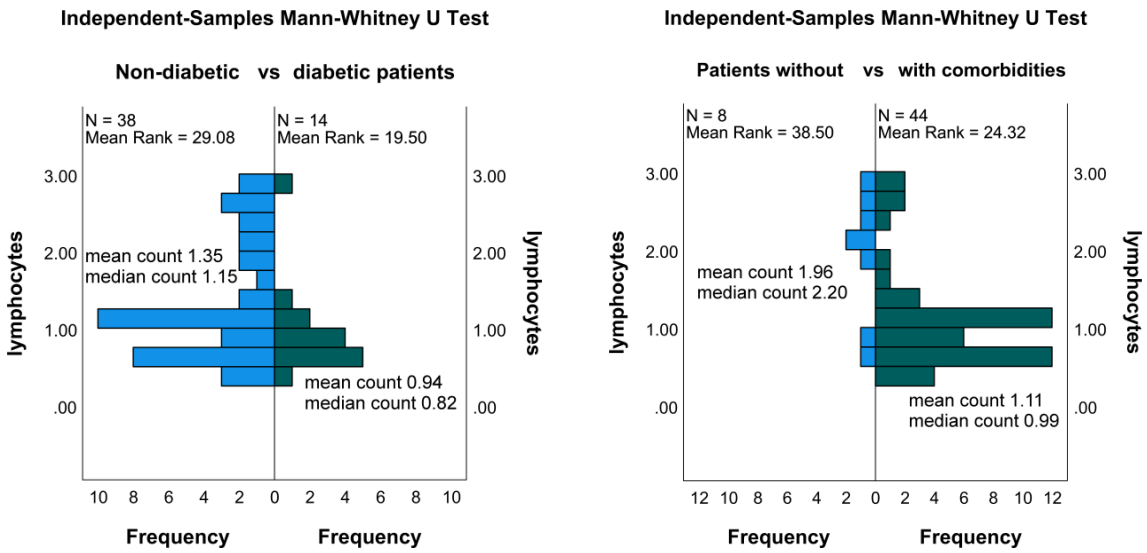
Correlation Matrix	WBC	neutrophils	lymphocytes	monocytes	eosinophils	basophils	CRP
WBC	1.000	.951	.198	.292	.062	-.173	.033
neutrophils	.951	1.000	-.047	.109	-.070	-.243	.051
lymphocytes	.198	-.047	1.000	.505	.303	.164	-.222
monocytes	.292	.109	.505	1.000	.182	.121	-.002
eosinophils	.062	-.070	.303	.182	1.000	.068	-.246
basophils	-.173	-.243	.164	.121	.068	1.000	-.290
CRP	.033	.051	-.222	-.002	-.246	-.290	1.000

MANOVA tests were used to determine whether any of the inflammatory markers, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, or CRP are linked to diabetes, obesity, or the presence of some general comorbidity (not including obesity). The results suggested that monocytes, lymphocytes, and basophils could be relevant.

Next, the post-hoc analysis was performed and the independent sample Mann-Whitney test was used to check for statistical significance ($p<0.05$) of the values of the three inflammation markers (monocytes, lymphocytes, and basophils) between COVID-19 positive patients without vs with diabetes, as well as without vs with comorbidities. This particular test can be used

with samples that are not paired and for which no assumptions are made about the shape of the data. No corrections for multiple comparisons have been applied.

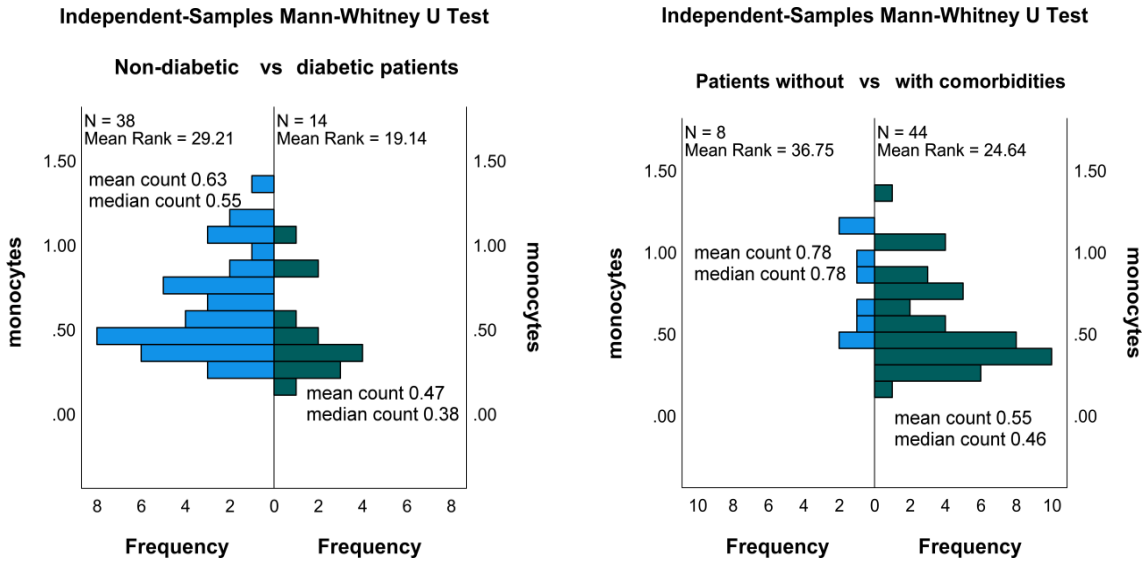
There was a statistically significant difference between the lymphocyte count depending on whether patients had diabetes or some comorbidity **(Figure 3)**. The count was higher for patients without diabetes and for patients without comorbidities, which agrees with previously published literature [35].



a) b)

Figure 3. Comparison of the lymphocyte count distributions in the Mann-Whitney U test. a) patients without vs with diabetes, with a p-value p=0.043. b) patients without vs with comorbidities, with a p-value p=0.013. The mean and median lymphocyte counts are shown in the figures. N is the number of patients in the corresponding subset.

There was a statistically significant difference between the monocyte count depending on whether patients had diabetes or some comorbidity **(Figure 4)**. The count was higher for patients without diabetes and for patients without comorbidities and was qualitatively similar to the result for lymphocytes which agrees with previously published literature [35]. This similarity in the qualitative behavior of lymphocytes and monocytes was consistent with the fact that they have the second highest correlation in **Table 1**.



a) b)

Figure 4. Comparison of the monocyte count distributions in the Mann-Whitney U test. a) patients without vs with diabetes, with a p-value p=0.034. b) patients without vs with comorbidities, with a p-value p=0.037. The mean and median monocyte counts are shown in the figures. N is the number of patients in the corresponding subset.

There was a statistically significant difference between the basophil counts depending on whether patients had some comorbidity **(Figure 5)**. Again, the count was higher for patients without comorbidities. A significance difference couldn't be established when classifying the patients by the presence of diabetes.

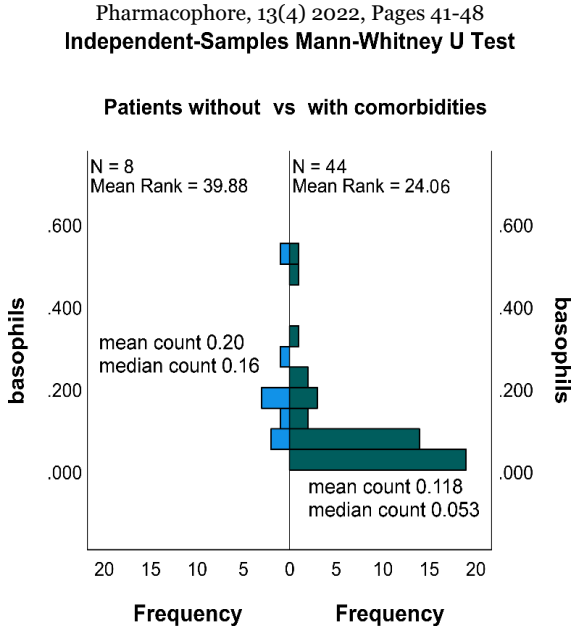


Figure 5. Comparison of the basophil count distributions in the Mann-Whitney U test for patients without vs with comorbidities, with a rather low p-value p=0.005. An outlier at 1.16 on the right panel has been removed only for better visualization purposes. The mean and median basophil counts are shown in the figure. N is the number of patients in the corresponding subset.

Other averages of inflammatory markers were also calculated, but they couldn't reach statistical significance when trying to differentiate between patients without and with diabetes or comorbidities **(Table 2)**.

Table 2. Average (avg.) and median (med.) values of various inflammatory markers as well as glycemia for patients without and with diabetes, comorbidities, or obesity.

Marker	Total avg. (med.)	Total std. dev.	Avg. (med.) for patients without diabetes	Avg. (med.) for patients with diabetes	Avg. (med.) for patients without comorbidities	Avg. (med.) for patients with comorbidities	Avg. (med.) for non-obese patients	Avg. (med.) for obese patients
WBC	12.7 (11.9)	4.68	12.2 (11.8)	13.7 (12.5)	12.9 (12.8)	12.6 (11.4)	12.8 (13.2)	12.5 (10.6)
Neutrophils	10.5 (10.1)	4.77	10.0 (9.7)	11.7 (10.7)	10.2 (10.7)	10.6 (9.8)	10.9 (10.9)	10.0 (8.1)
Lymphocytes	1.24 (1.02)	0.75	1.35 (1.15)	0.94 (0.82)	1.96 (2.20)	1.11 (0.99)	1.22 (0.90)	1.26 (1.06)
Monocytes	0.59 (0.48)	0.29	0.63 (0.55)	0.47 (0.38)	0.78 (0.78)	0.55 (0.46)	0.57 (0.48)	0.60 (0.48)
Eosinophils	0.065 (0.113)	0.15	0.077 (0.017)	0.032 (0.006)	0.075 (0.041)	0.063 (0.009)	0.049 (0.01)	0.085 (0.014)
Basophils	0.13 (0.07)	0.19	0.12 (0.079)	0.16 (0.05)	0.20 (0.16)	0.118 (0.053)	0.148 (0.064)	0.11 (0.074)
CRP	149.8 (137.8)	102.6	149.6 (136.5)	150.1 (139.7)	158.1 (147.1)	148.4 (137.1)	127.6 (137.1)	194.2 (141.6)
Glycemia	160.5 (132.0)	84.0	143.6 (126.0)	207.4 (162.0)	122.8 (118.0)	165.8 (139.0)	155.9 (144.0)	165.8 (132.0)

The averages for glycemia were also added in **Table 2**. An independent sample Mann-Whitney test revealed a statistically significant difference in the glycemia of non-diabetic vs diabetic patients (p=0.024). The average glycemia was rather high for all patients, a fact that has been reported before in the literature [35].

Conclusion

The inflammatory parameters such as WBC, neutrophils, and CRP presented high levels in patients with COVID-19, and so was their glycemia, which was even higher ($p<0.05$) in diabetic patients. Large neutrophil/lymphocyte and low lymphocyte/CRP ratios suggested moderate to severe disease for these patients. The correlation between hematological markers was also calculated, and while WBC and neutrophils were highly correlated due to the dominating number of neutrophils, the next largest correlation was found between lymphocytes and monocytes. Statistical analysis (using the $p<0.05$ criterion) showed that patients without comorbidities had higher values of lymphocytes, monocytes, and basophils than those with comorbidities, and non-diabetic patients had higher values of lymphocytes and monocytes than diabetic ones.

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Conflict of interest: None

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Ethics statement: The data about the patients with COVID-19 was collected from the County Clinical Emergency Hospital of Oradea through online access (permission nr. 10538/04.04.2022). Permission from the Ethical Committee of the Faculty of Medicine and Pharmacy, University of Oradea was also obtained (CEFMF/08/30.05.2022).

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