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Analysis of complete blood count and C-reactive protein with respect to COVID-19 patients co-infected with fungi in Anbar, Iraq

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Keywords: CBC, N/L, CRP, COVID-19, Fungi.

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Abstract:

COVID-19 exhibits a systemic inflammatory response to heightened blood levels of complete blood count (CBC) and C-reactive protein (CRP) that are indicative of severe illness in microbial (bacteria and viruses) infections. We aimed to investigate the correlation between CBC and CRP levels during the first hospitalization and clinical outcomes in COVID-19 patients with fungal co-infection. This research involved 100 post-COVID-19 patients referred to Al-Shafaa Hospital in Al-Ramadi, Anbar province, from October 2022 to May 2023. Each patient had blood drawn to determine their total blood count and C-reactive protein titer. Even though only nine patients had high CRP levels, the current study found that patients had insignificantly ($P< 0.05$) higher CRP levels than controls. It was revealed that 39 out of 100 patients developed an elevated white blood cell count. In contrast, five patients acquired a high RBC count. Neutrophilia was found in 39 of the patients. Five patients developed lymphocytopenia. In contrast, 12 patients had a high lymphocyte count. CRP and N/L ratio significantly discriminate patients from control. In conclusion, the results of CBC and CRP were variable among COVID-19 patients co-infected with fungi. Nevertheless, CRP and N/L markers are reliable and sensitive predictors of clinical outcomes in patients with COVID-19.

Keywords: CBC, N/L, CRP, COVID-19, Fungi.

تحليل تعداد الدم الكامل والبروتين سي التفاعلي فيما يتعلق بمرضى كوفيد-١٩ المصابين بالفطريات في الأنبار، العراق

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الخلاصة:

يُظهر كوفيد-١٩ استجابة التهابية جهازية لارتفاع مستويات الدم في تعداد الدم الكامل (*CBC*) والبروتين التفاعلي (*CRP*) مما يدل على مرض شديد في العدوى الميكروبية (البكتيريا والفيروسات). نحن نهدف إلى دراسة العلاقة بين مستويات *CBC* و*CRP* أثناء الاستشفاء الأول والنتائج السريرية لدى مرضى كوفيد-١٩ الذين لديهم أيضًا عدوى فطرية مصاحبة. شمل هذا البحث ١٠٠ مريض ما بعد كوفيد-١٩ أُحيلوا إلى مستشفى الشفاء في الرمادي بمحافظة الأنبار، في الفترة من أكتوبر ٢٠٢٢ إلى مايو ٢٠٢٣. وتم سحب دم من كل مريض لتحديد تعداد الدم الإجمالي وعيار البروتين التفاعلي سي. على الرغم من أن تسعة مرضى فقط لديهم مستويات عالية من *CRP*، فقد وجدت الدراسة الحالية أن المرضى لديهم مستويات *CRP* أعلى بشكل ملحوظ ($P < 0.05$) من الضوابط. وكشف أن ما مجموعه ٣٩ من أصل ١٠٠ مريض أصيبوا بارتفاع عدد خلايا الدم البيضاء. في المقابل، حصل ٥ مرضى على عدد كرات الدم الحمراء مرتفع. تم العثور على العدلات في ٣٩ من المرضى. أصيب خمسة مرضى بنقص اللمفاويات. في المقابل، كان لدى ١٢ مريضا ارتفاع في عدد الخلايا الليمفاوية. نسبة *CRP* و*N/L* تميز بشكل كبير المرضى عن السيطرة. في الختام: كانت نتائج *CBC* و*CRP* متباينة بين مرضى كوفيد-١٩ المصابين بالفطريات. ومع ذلك، تعد كل من علامات *CRP* و*N/L* بمثابة تنبؤات موثوقة وحساسة للنتائج السريرية لدى المرضى المصابين بـ*COVID-19* .

الكلمات المفتاحية: تعداد الدم الكامل، *N/L*، الالتهاب بروتيني التفاعلي، كوفيد-١٩، الفطريات.

1. INTRODUCTION:

SARS-CoV-2 is an enveloped virus with a diameter of 100 nm and a mass of approximately one femtogram. Its genome consists of a linear single-stranded RNA, positive-sense with a length of about 29,800 base pairs, with ⅔ encoding non-structural proteins and one-third encoding structural proteins, including membrane (M), envelope (E), nucleocapsid (N), and spike (S) proteins [1].

The liver produces C-reactive protein (CRP) upon the action of IL-6, and it is commonly used as a bioindicator of inflammation [2-4]. Multiple studies have established a link between elevated CRP levels and increased disease severity in COVID-19 patients, as well as in individuals with H1N1 influenza pneumonia [5]. COVID-19 is known to cause noticeable

demonstrations in the hematopoietic system, with common hematological abnormalities recognized in affected individuals. Platelets, lymphocytes, hemoglobin, eosinophils, and basophils have all exhibited marked decreases from the disease's initial stages, and these changes have been associated with disease severity and clinical outcomes. The growth and division of monocytes throughout the infection course of COVID-19 remain indeterminate, given that the SARS-CoV-2 infection appears to directly weaken adaptive immune responses against viruses. Moreover, an elevation in the level of neutrophils and the neutrophil-to-lymphocyte ratio (NLR) has been linked to progressive disease. The assessment of laboratory data at baseline and throughout the infection process may aid scientists in developing custom-made treatment approaches as well as providing thorough care to those who require it the most **[6]**.

In this study, we aim to investigate the relationship between CRP concentrations at hospital admission and pathological findings in COVID-19 patients in Ramadi, Iraq, in a healthcare system of considerable size.

2. Materials and Methods:

2.1. Study group

A total of 100 post-COVID-19 patients coinfected with fungi (*Aspergillus and Candida*) referring to Al-Shafaa Hospital in Al-Ramadi, Anbar province were enrolled in this study for the period October 2022 to May 2023. Blood specimens were collected from each patient to assay the complete blood count and C-reactive protein titer.

2.2. Blood assays

2.2.1. Complete Blood Count

XN 350 Sysmex hematology analyzer was used to perform this test.

2.2.2. C-Reactive Protein

C-reactive protein titer was estimated as instructed by the manufacturer company (Fine Care, UK).

3. Statistical analysis

All experiments were performed in triplicate and data are expressed as mean and standard deviation. Kolmogorov-Smirnov and Shapiro-Wilk tests were performed to test the normality distribution of data. Categorical data were demonstrated as numbers (percentage); whereas the nonparametric data were demonstrated as median. Mann-Whitney and Kruskal-Wallis tests were employed to evaluate the differences among study groups medians. Chi-square was used to test the demographic parameters. Regarding CRP, the receiver operating curve (ROC)

analysis was performed to calculate the cut-off value, sensitivity, and specificity. The differences were considered significant when the P value ≤ 0.05. These statistical analyses were done using GraphPad Prism 9.5.0 software.

4. Results and Discussion:

4.1. Frequency of CRP by co-infection with fungi

Even though only nine patients demonstrated high levels of CRP (>10 mg/L), the current study showed that patients developed insignificantly (P< 0.05) higher (6.624 ± 3.914 mg/L) CRP levels than controls (4.544 ± 1.9 mg/L), as it is presented in **Fig.1**.

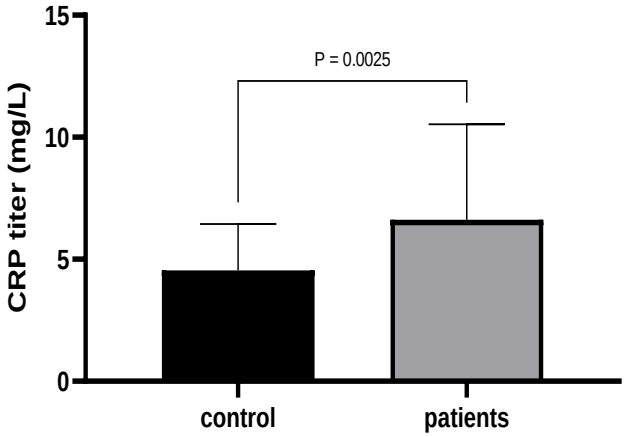

Fig. 1: Level of CRP in control vs. patients of COVID-19 presented with fungal infection. Mann-Whitney test P < 0.05.

Although the area under the curve (AUC) is 0.691 ± 0.051 (CI95% = 0.59 - 0.79, P= 0.03), CRP cannot discriminate the infection with *Aspergillus* or *Candida* with a cut-off value = 6.95 mg/L, due to the low sensitivity (0.384) but it can detect the negative cases very accurately (specificity = 0.96) as it is depicted in **Fig. 2**.

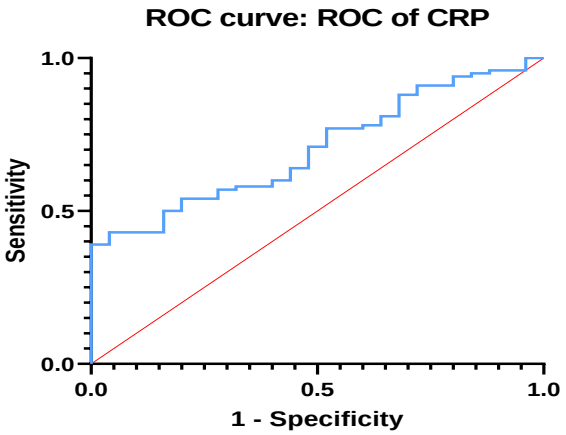

Fig. 2: Receiver operating characteristic (ROC) curve of CRP, AUC = 0.693, CI95% = 0.59-0.79, P= 0.028, Cut off value = 6.95, sensitivity = 0.384, specificity = 0.96

The CRP level significantly increased (P<0.05) compared to controls. Additionally, the CRP level exhibited a greater increase in the infection initial phase among the patient group (p<0.01). Nevertheless, CRP concentration in bacterial infections insignificantly differed (p > 0.05) from the fungal infection during the infection acute phase [7].

CRP has been shown to stimulate phagocytosis by phagocytes through a specific CRP receptor, which aids in the removal of various pathogenic microorganisms. A cytokine storm may be induced in COVID-19 pneumonia, which is linked with a significant death risk. Cytokines including IL-6 and TNF-α may drive the liver to create CRP, a biomarker that closely correlates with COVID-19 development and is highly increased during the early phases of inflammation. Systemic inflammation, in terms of CRP, is highly linked to venous thromboembolism, acute kidney injury, serious diseases, and hospital-related death rates in COVID-19 patients. Hence, evaluating inflammatory bioindicator-based methods for risk classification and treatment could improve patient outcomes [8, 9]. Regarding baseline laboratory data, the fungal positive group exhibited statistically significant differences in CRP, D-dimer, and serum ferritin levels (P< 0.05) compared to the fungal negative group, on the contrary, the CBC indices differed insignificantly (P> 0.05) [10].

4.2. Complete blood count according to co-infection with fungi

The result of CBC is grouped in Table 1.

Table 1: Frequencies of WBC total and differential count, RBC, hemoglobin, and platelets in patients of COVID-19 according to fungal isolates

Criterion	*C. albicans*	*C. tropicalis*	*A. flavus*	*A. niger*	Total
WBC (< 4 × 10^9 cell/L)	0	0	0	0	0
WBC (> 11 × 10^9 cell/L)	20	8	6	3	39
Neutrophil (< 1.6 × 10^9 cell/L)	0	0	0	0	0
Neutrophil (> 7 × 10^9 cell/L)	21	7	7	4	39
Eosinophil (< 0.001 × 10^9 cell/L)	0	0	0	0	0
Eosinophil (> 0.4 × 10^9 cell/L)	0	2	0	0	2
Basophil (< 0.001 × 10^9 cell/L)	0	0	0	0	0
Basophil (> 0.8 × 10^9 cell/L)	0	0	0	0	0
Lymphocytes (< 0.8 × 10^9 cell/L)	5	0	0	0	5
Lymphocytes (> 4 × 10^9 cell/L)	6	4	2	0	12
Neutrophil/lymphocyte ratio	16	3	5	3	27
Monocytes (< 0.24 × 10^9 cell/L)	1	0	0	0	1
Monocytes (> 0.7 × 10^9 cell/L)	43	11	9	6	69
RBC (< 4 × 10^{12} cell/L)	8	0	0	1	9
RBC (> 5.5 × 10^{12} cell/L)	2	2	0	1	5
Haemoglobin (< 11 g/dL)	15	2	3	1	21
Haemoglobin (> 16 g/dL)	3	0	0	2	5
Platelets (< 1.5 × 10^{11} cell/L)	5	0	1	0	6
Platelets (> 4.5 × 10^{11} cell/L)	3	1	2	0	6

4.3. WBC total and differential count

The findings of the current work illustrated in **Fig. 3** revealed that a total of 39 out of 100 patients developed high white blood cell count (> 11 × 10^9 cell/L); 20, 8, 6, and 3 of them were infected with *C. albicans*, *C. tropicalis*, *A. flavus*, and *A. Niger*, respectively. WBC levels in the fungal-infected group dropped during the beginning infection and acute phases when compared to the control group ($p < 0.01$) **[7]**.

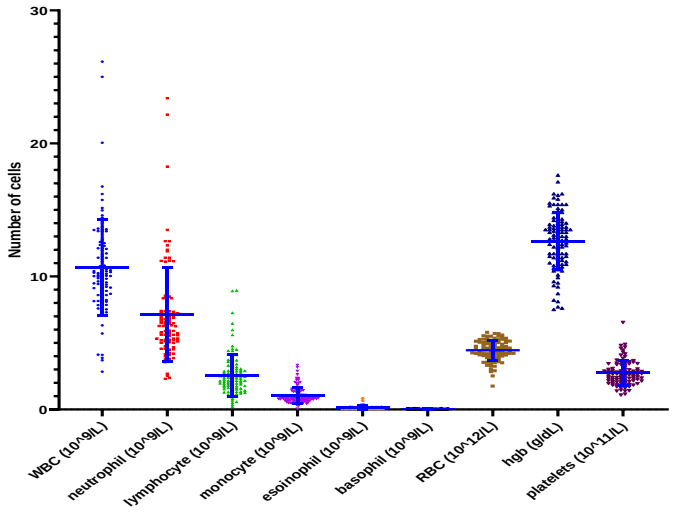

Fig. 3: Scatter plot of the frequencies of WBC total and differential count, RBC, hemoglobin, and platelets in patients of COVID-19 co-infected with *Aspergillus* or *Candida*. Horizontal bars represent mean ± standard deviation.

Neutrophilia was observed in 39 patients; 21, 7, 7, and 4 were co-infected with *C. albicans*, *C. tropicalis*, *A. flavus*, and *A. Niger*, respectively. Two of the patients who developed eosinophilia were co-infected with *C. tropicalis*. None of the patients developed basophilia.

Neutrophils play a crucial role in defending against fungal pathogens by migrating toward the invading microorganisms and eliminating them through phagocytosis, oxidative burst, and the release of neutrophil extracellular traps **[11]**. Meanwhile, there have been varying reports on eosinophil counts during COVID-19, and it remains unclear whether these changes are related to the primary disease process or a result of treatment-induced immunomodulation **[12]**. The microbial (bacteria, fungi, and viruses) cytoplasmic membrane contains phospholipids, which interact with the CD300a receptor on eosinophils **[13]**.

Five patients developed lymphocytopenia (< 0.8 × 10^9 cell/L) all of them were co-infected with *C. albicans*. While 12 patients showed high lymphocyte count (> 4 × 10^9 cell/L), 6, 4, and 2 were co-infected with *C. albicans*, *C. tropicalis*, and *A. flavus*, respectively. Regarding monocytes, one patient developed monocytopenia (< 0.24 × 10^9 cell/L) and was co-infected

with *C. albicans*. While, 69 patients showed high monocyte count (> 7×10^9 cell/L), 43, 11, 9, and 6 were co-infected with *C. albicans*, *C. tropicalis*, *A. flavus*, and *A. Niger*, respectively.

Both nonspecific innate and acquired immunity, including neutrophils, macrophages, dendritic cells, lymphocytes, and monocytes, are important in host defense against congenital mycoses. Th1 lymphocytes secrete cytokines such as IFN-γ, IL-2, and IL-12, which induce cytotoxic cells and neutrophils to abolish fungal cells. Th2 lymphocytes, on the other hand, secrete cytokines such as IL-4, IL-6, and IL-10, which reduce cellular immunity by counter-regulating the production of IL-2, IL-12, and IFN- γ and depressing macrophage activity. Cellular processes are also important in host responses to fungal infections, and T-lymphocyte malfunction and reduction in number are common in individuals with mycotic illnesses **[14]**. Monocytes and their descendants, such as macrophages and dendritic cells, play a variety of functions in the immune response to fungi. Monocytes detect fungus and activate signaling pathways that cause direct actions like as phagocytosis and cytokine generation. Monocytes are additionally able to deliver fungal antigenic components to generate adaptive immune responses **[15]**.

The present results revealed that 16, 3, 5, and 3 patients co-infected with *C. albicans*, *C. tropicalis*, *A. flavus*, and *A. Niger*, respectively developed an N/L ratio above the control mean (4.435). C. albicans patients significantly outnumbered other patients.

Receiver operating analysis demonstrated a cut-off of 3.135 (AUC = 0.671, CI95% = 0.569-0.773, P= 0.008, sensitivity = 0.59, specificity = 0.76) for neutrophil/lymphocytes ratio (NLR) as depicted in **Fig. 4**.

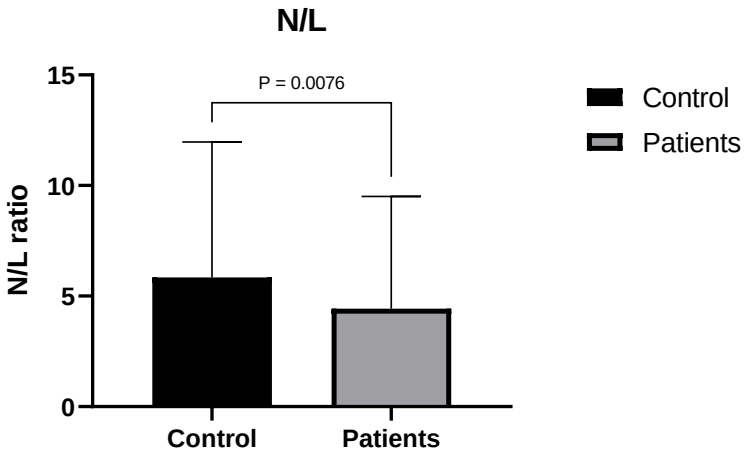

Fig. 4: N/L ratio in control vs. patients of COVID-19 presented with fungal infection. Mann-Whitney test P < 0.05.

Combining the NLR with the age variable can aid in risk stratification and guide the establishment of diagnostic and therapeutic procedures for COVID-19 patients. A high neutrophil-to-lymphocyte ratio indicates a poorer chance of survival, making risk categorization and management crucial for alleviating medical resource shortages and reducing the mortality rate of critically ill patients **[16]**.

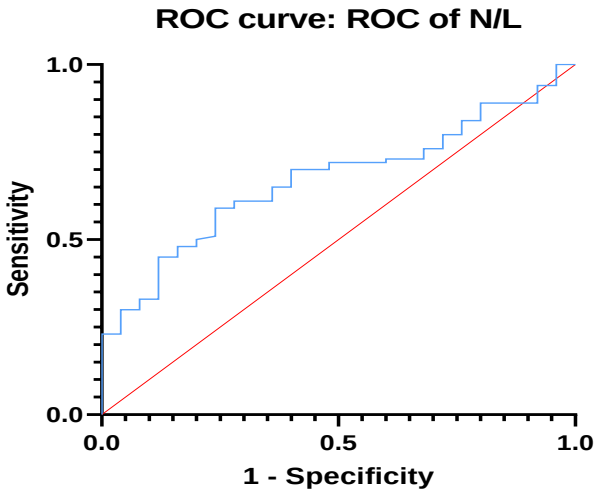

Fig. 5: Receiver operating characteristic (ROC) curve of N/L ratio, AUC = 0.671, CI95% = 0.569-0.773, P= 0.008, Cut off value = 3.135, sensitivity = 0.59, specificity = 0.76

4.4. RBC and hemoglobin

Regarding RBC, it was found that 9 patients had low RBC count (< 4×10^{12} cell/L), 8 of them infected with *C. albicans* and one patient with *A. Niger*. On the other hand, 5 patients developed high RBC count (> 5.5×10^{12} cell/L), 2, 2, and 1 patient were co-infected with *C. albicans*, *C. tropicalis*, and *A. Niger*, respectively. Concerning the hemoglobin results, it was found that 21 and 5 patients developed anemia (< 11 g/dL) and high (> 16 g/dL) hemoglobin concentration. Furthermore, 15, 2, 3, and 1 of those who developed low hemoglobin were co-infected with *C. albicans*, *C. tropicalis*, *A. flavus*, and *A. Niger*; whereas 3 and 2 of those presented with high hemoglobin levels were co-infected with *C. albicans* and *A. Niger*.

4.5. Platelets

The present findings revealed that 6 patients developed low platelet counts and another 6 developed high counts (**Fig. 5**). Out of the thrombocytopenia patients, 5 and 1 of them were co-infected with *C. albicans* and *A. flavus*, respectively. Nevertheless, 3, 1, and 2 patients with increased platelets were co-infected with *C. albicans*, *A. flavus*, and *A. Niger*, respectively.

Platelet count was reduced in the fungal-infected group in comparison to the control in the initial and acute phases of infection (P< 0.01). Platelet count was reduced in the bacterial-

infected group during the infection's initial and acute phases ($p < 0.01$). Furthermore, the fungal infection group had a greater decline in platelets number than the bacterial infected group ($P<0.01$); nevertheless, in the infection acute phase, platelets count differs insignificantly between bacterial- and fungal-infected groups ($P > 0.05$) [7].

5. Conclusions

CBC and CRP levels varied among COVID-19 patients who were also afflicted with pulmonary aspergillosis and candidiasis. CRP and N/L indicators are similarly accurate and sensitive for predicting COVID-19 in-hospital outcomes.

Conflict of interests

The authors declare that there is no conflict of interest.

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