Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

International Immunopharmacology 100 (2021) 108137

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

A possible pathogenic correlation between neutrophil elastase (NE) enzyme and inflammation in the pathogenesis of coronavirus disease 2019 (COVID-19)

Sajad Karampoor [a,b,1,*], Khashayar Hesamizadeh [b,c,1], Faezeh Maleki [d], Mohammad Farahmand [e], Hamid Zahednasab [f], Rasoul Mirzaei [g], Helene Banoun [h], Farhad Zamani [a], Marzieh Hajibaba [a], Alireza Tabibzadeh [b], Behnaz Bouzari [i], Mohammad-Navid Bastani [b], Azadeh Laali [j], Hossein Keyvani [b,a,*]

[a] Gastrointestinal and Liver Diseases Research Center, Iran University of Medical Sciences, Tehran, Iran
[b] Department of Virology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[c] Middle East Liver Diseases (MELD) Center, Tehran, Iran
[d] Human Viral Vaccine Department, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Alborz Province, Iran
[e] Department of Medical Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[f] Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
[g] Department of Microbiology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
[h] Independent researcher, Former research fellow at INSERM (French Institute for Health and Medical Research), Marseille, France
[i] Department of Pathology, Firouzgar Hospital, Iran University of Medical Sciences, Tehran, Iran
[j] Department of Infectious Disease, School of Medicine, Firoozgar General Hospital, Iran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
COVID-19
SARS-CoV-2
Neutrophil elastase
Inflammation
Disease activity

ABSTRACT

A growing body of evidence indicates that neutrophil elastase (NE) is involved in the pathogenesis of respiratory infectious diseases, such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This study aimed to analyze the dynamic changes in serum levels of NE associated with inflammation, disease activity, and mortality rate in patients with COVID-19. We measured the serum concentrations of NE, C-Reactive protein (CRP), interleukin (IL)- 4, IL-6, IL-8, IL-10, and vitamin D levels in 83 ICU and 69 non-ICU patients compared with 82 healthy subjects (HS) in three-time points (T1-T3). Serum levels of NE, IL-6, IL-8, and CRP in ICU and non-ICU patients were significantly higher than HS ($P < 0.001$) in three-time points. Also, serum levels of NE, IL-6, IL-8, and CRP in ICU patients were significantly higher than in non-ICU patients ($P < 0.05$). On the day of admission (T1), the levels of NE, CRP, IL-6, IL-8 were gradually decreased from T1 to T3. At the same time, IL-4 and IL-10 were gradually increased from T1 to T2 and then reduced to T3. Further analyses demonstrated that the levels of NE, IL-6, and IL-8 in deceased patients were significantly higher than in recovered patients ($P < 0.05$). The ROC curve analysis demonstrated that markers, including NE, IL-6, and IL-8, were valuable indicators in evaluating the activity of COVID-19. Overall, our results signify the critical role of NE in the pathogenesis of COVID-19, and also, further support that NE has a potential therapeutic target for the attenuation of COVID-19 severity.

1. Introduction

Recently, the pandemic coronavirus called SARS-CoV-2 prompted an intimidating disease, which is characterized by high mortality, economic, and well-being burden on the world society [1–3]. SARS-CoV-2 is the causative agent of COVID-19, which was first identified in Wuhan, Hubei province, China, and then was spread worldwide [4–7]. In addition to its critical respiratory destruction, COVID-19 provoked thromboembolic issues and multi-organ collapse, including kidneys, heart, and liver [8,9]. These outcomes may be due to overactive innate

* Corresponding authors.
E-mail addresses: karampour.s@iums.ac.ir (S. Karampoor), keyvani.h@iums.ac.ir (H. Keyvani).
[1] contributed equally to this work.

https://doi.org/10.1016/j.intimp.2021.108137
Received 6 July 2021; Received in revised form 18 August 2021; Accepted 5 September 2021
Available online 14 September 2021

Table 1
Demographic and laboratory markers of patients with COVID-19 and HS.

Variable	N	Overall, N = 234[1]	Group			p-value[2]
			Control, N = 82[1]	ICU, N = 83[1]	Non-ICU, N = 69[1]	
Sex	234					0.011
Female		98 (42%)	34 (41%)	44 (53%)	20 (29%)	
Male		136 (58%)	48 (59%)	39 (47%)	49 (71%)	
Age	234	54 (37, 65)	46 (36, 59)	58 (40, 70)	53 (36, 63)	0.006
CRP_T1	234	79 (2, 100)	2 (1, 2)	100 (85, 127)	84 (78, 100)	<0.001
IL8_T1	209	145 (49, 167)	32 (23, 43)	168 (146, 202)	146 (139, 156)	<0.001
IL6_T1	234	59 (23, 78)	21 (19, 24)	86 (68, 114)	63 (56, 72)	<0.001
IL4_T1	234	2.48 (1.30, 3.10)	1.20 (0.90, 1.30)	3.00 (2.50, 3.25)	2.80 (2.45, 3.14)	<0.001
IL10_T1	234	4.0 (3.0, 5.0)	3.0 (2.2, 3.2)	5.0 (4.0, 7.0)	4.0 (3.0, 5.1)	<0.001
Elastas_T1	234	178 (105, 192)	93 (84, 112)	195 (187, 204)	180 (169, 189)	<0.001
VitD_T2	151	18 (15, 21)	NA (NA, NA)	17 (15, 21)	18 (15, 21)	0.8

[1] n (%); Median (IQR)
[2] Pearson's Chi-squared test; Kruskal-Wallis rank sum test

immune reactions [10] and a cytokine storm generated by lymphocyte and macrophage activation induced by SARS-CoV-2 [11,12]. The neutrophils work as an essential component of the immune defense barrier linking innate and adaptive immunity among the innate immune cells. They cooperate to remove exogenous pathogens and endogenous cell debris and perform a crucial function in the pathogenesis of numerous respiratory disorders, such as viral respiratory infections [13–16].

Neutrophils have an armory of defensive tactics that comprise neutrophil extracellular traps (NETs), discharge of antimicrobial granules, and NE (a serine protease) [13,17]. The histone-DNA ingredients of falling neutrophils are described as NETs, which are involved in host protection against pathogens [13,17]. An investigation reported that two markers of NETs, including cell-free DNA and myeloperoxidase (MPO)-DNA, were elevated in hospitalized COVID-19 cases compared with 30 controls and related to CRP, D-dimer, lactate dehydrogenase, and total neutrophil number [18]. Besides, in recent work performed on ambulatory cases, it has been found that myeloperoxidase-DNA concentration is increased in the initial phase of SARS-CoV-2 infection [19]. Also, another crucial antimicrobial compound released from neutrophils is NE, which is thought to possess antimicrobial properties either by its proteolytic activity or the production of ROS [20]. NE is found in the serum and airways in hospitalized infants infected with the respiratory syncytial virus (RSV) [21,22]. This finding indicates that during RSV infection, activated neutrophils are recruited and enter the lung. However, the putative function of neutrophils in prophylaxis versus pathogenesis is still opaque. [23]. A recent study in the field of inflammation indicated that air hydrogen sulfide stimulates macrophage extracellular traps to exacerbate inflammatory damage via the control of miR-15b-5p on MAPK and insulin signals in the trachea of chickens [24]. Another finding showed that environmental factors including lithium [25] and contaminant ammonia [26] promote the occurrence of inflammatory responses.

Further evidence has highlighted the crucial function of NE in pathologic conditions, including chronic obstructive pulmonary disease

Table 2
Demographic and clinical parameters of ICU and non-ICU patients.

Variable	N	Overall, N = 152[1]	Group		p-value[2]
			ICU, N = 83[1]	Non-ICU, N = 69[1]	
Sex	152				0.003
Female		64 (42%)	44 (53%)	20 (29%)	
Male		88 (58%)	39 (47%)	49 (71%)	
Age	152	56 (38, 68)	58 (40, 70)	53 (36, 63)	0.047
Platelet	152	175 (140, 232)	196 (160, 234)	154 (119, 227)	0.006
MCH	152	27.9 (25.0, 29.9)	29.0 (27.0, 30.4)	25.7 (23.7, 28.2)	<0.001
MCV	152	87 (83, 91)	88 (85, 91)	86 (79, 90)	0.007
Hb	152	12.75 (10.60, 14.43)	13.00 (11.35, 14.40)	12.40 (9.70, 14.50)	0.3
Hct	152	40 (34, 43)	40 (34, 44)	40 (36, 43)	>0.9
WBC	152	6.30 (5.10, 8.65)	5.90 (4.85, 9.05)	6.30 (5.40, 8.20)	0.6
LDH	152	453 (374, 650)	576 (456, 708)	420 (345, 452)	<0.001
SGPT	152	19 (15, 32)	23 (16, 36)	17 (13, 22)	<0.001
SGOT	152	24 (18, 41)	27 (19, 45)	23 (17, 26)	0.013
Cigarette	152				0.014
Negative		132 (87%)	67 (81%)	65 (94%)	
Positive		20 (13%)	16 (19%)	4 (5.8%)	
Opium	152				>0.9
Negative		148 (97%)	81 (98%)	67 (97%)	
Positive		4 (2.6%)	2 (2.4%)	2 (2.9%)	
Alcohol	152				>0.9
Negative		145 (95%)	79 (95%)	66 (96%)	
Positive		7 (4.6%)	4 (4.8%)	3 (4.3%)	
Kidney. failure	152				0.10
Negative		131 (86%)	68 (82%)	63 (91%)	
Positive		21 (14%)	15 (18%)	6 (8.7%)	
Dialysis	152				0.5
Negative		141 (93%)	78 (94%)	63 (91%)	
Positive		11 (7.2%)	5 (6.0%)	6 (8.7%)	
Lung. disease	152				0.2
Negative		131 (86%)	69 (83%)	62 (90%)	
Positive		21 (14%)	14 (17%)	7 (10%)	
Heart. disease	152				0.6
Negative		127 (84%)	68 (82%)	59 (86%)	
Positive		25 (16%)	15 (18%)	10 (14%)	
Discharg	152	7 (5, 8)	8 (7, 9)	6 (5, 6)	<0.001
Death	152				0.002
Negative		142 (93%)	73 (88%)	69 (100%)	
Positive		10 (6.6%)	10 (12%)	0 (0%)	
CRP_T1	152	90 (79, 120)	100 (85, 127)	84 (78, 100)	<0.001
CRP_T2	152	43 (34, 48)	43 (35, 52)	40 (33, 46)	0.015
CRP_T3	152	22 (19, 26)	22 (18, 26)	23 (19, 26)	0.7
IL8_T1	152	155 (140, 174)	168 (146, 202)	146 (139, 156)	<0.001
IL8_T2	152	204 (184, 276)	267 (212, 301)	188 (178, 199)	<0.001
IL8_T3	152	101 (87, 116)	112 (96, 126)	90 (80, 102)	<0.001
IL6_T1	152	72 (59, 90)	86 (68, 114)	63 (56, 72)	<0.001
IL6_T2	151	98 (87, 123)	107 (89, 144)	90 (87, 101)	<0.001
IL6_T3	151	43 (33, 53)	41 (32, 51)	44 (36, 53)	0.3
IL4_T1	152	3.00 (2.50, 3.20)	3.00 (2.50, 3.25)	2.80 (2.45, 3.14)	0.3
IL4_T2	152	4.71 (4.43, 5.40)	5.23 (4.56, 5.43)	4.56 (4.33, 5.10)	<0.001
IL4_T3	151	2.80 (2.44, 3.10)	3.00 (2.60, 3.20)	2.50 (2.39, 2.89)	<0.001
IL10_T1	152	5.0 (3.7, 6.0)	5.0 (4.0, 7.0)	4.0 (3.0, 5.1)	0.012
IL10_T2	151	8 (6, 14)	13 (8, 23)	6 (5, 8)	<0.001
IL10_T3	152	7 (5, 10)	9 (6, 14)	5 (4, 7)	<0.001
Elastas_T1	152				<0.001

(*continued on next page*)

Table 2 (*continued*)

Variable	N	Overall, N = 152[1]	Group		p-value[2]
			ICU, N = 83[1]	Non-ICU, N = 69[1]	
		189 (178, 199)	195 (187, 204)	180 (169, 189)	
Elastas_T2	151	165 (158, 176)	172 (166, 182)	158 (152, 163)	<0.001
Elastas_T3	151	136 (130, 142)	139 (134, 145)	134 (128, 137)	<0.001
VitD_T1	152	17 (13, 21)	17 (13, 21)	18 (14, 23)	0.2
VitD_T2	151	18 (15, 21)	17 (15, 21)	18 (15, 21)	0.8
VitD_T3	151	20 (16, 22)	21 (16, 22)	18 (16, 21)	0.4
Ct_value	151	29 (24, 33)	25 (24, 33)	32 (24, 34)	0.15

[1] n (%); Median (IQR)
[2] Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test

and lung injury [27–29]. Besides, recently, it has been noticed that NE may be implicated in the pathogenesis of COVID-19 [30]. Therefore, the present study evaluated the association between dynamic changes in NE levels associated with inflammatory markers (IL-6 and IL-8), anti-inflammatory markers (IL-4, IL-10), and vitamin D levels on the three-time points (T1-T3) in ICU and non-ICU COVID-19 patients.

2. Methods

2.1. Subjects

A case-control study was intended to analyze whether the dynamic alterations in the levels of NE are associated with dynamic changes in the levels of inflammatory markers (CRP, IL-6, and IL-8), anti-inflammatory markers (IL-4 and IL-10), and vitamin D levels in ICU and non-ICU patients with COVID-19 in T1 to T3. Also, we addressed the correlation of dynamic changes in these factors with the disease severity. In this study, COVID-19 patients were recruited and referred to Firouzgar Hospital, Tehran, Iran, between November 2020 and April 2021. In order to obtained the legal and ethical authority for collecting the specimens, informed consent was obtained from all individuals who participated in this study. Additionally, this research was confirmed by the ethics committee of the Iran University of Medical Sciences (IUMS) (ECIUMS; IR.IUMS.REC.1399.175). A total number of 152 patients with COVID-19 were admitted to Firouzgar Hospital and classified into two groups according to the criteria established by Li and colleagues; the first group comprised 83 cases with COVID-19 (severe patients hospitalized in ICU), the second group consisted of 69 patients with COVID-19 (moderate patients). Also, in this study, 82 healthy subjects were enrolled as the control group.

2.2. Laboratory validation and treatment

The study was performed on three time points as follows: T1: day of hospitalization, T2: 3 days after hospitalization, T3: 6 days after hospitalizations. At each time point, 5 ml peripheral blood was collected from each patient and quickly following the sample gathering, serum samples were isolated by centrifugation and kept at − 70C° until use. For RNA extraction from sputum and throat swab specimens, the QIAamp Viral RNA Mini Kit (Qiagen, Germany) was utilized according to the manufacturer's instructions. Then, the real-time RT-PCR assay was applied for the detection of RNA of SARS-CoV-2. The routine biochemical parameters of sera obtained from confirmed COVID-19 patients were analyzed on the day of admission by standard automated methods in a Technicon Dax autoanalyzer (Technicon Instruments, CO, USA).

2.3. The enzyme-linked immunosorbent assay

The levels of inflammatory (CRP; BOSTER BIOLOGICAL TECHNOLOGY, EK7040, IL-6; Abcam # ab46027, IL-8; Abcam # ab46032), and anti-inflammatory (IL-4; IBL International GmbH # BE58041, IL-10; IBL International GmbH # BE58101) markers were assessed according to the manufacturer's structure. We used a quantitative chemiluminescent immunometric assay for the assessment of Vit D levels in serum specimens (DiaSorin, spA, Via Crescentino, Vercelli, Italy). All specimens were examined in duplicate, and the mean values were used for the statistical interpretations.

2.4. Statistical methods

Continuous and categorical variables were presented as median (IQR) and n (%), respectively. In order to compare the differences between experimental groups, the Wilcoxon rank-sum test, $\chi 2$ test, or Fisher's exact test were applied, where appropriate. In addition, the relationship between laboratory measurements was calculated using the Pearson correlation coefficient. Regarding the total number of Non-ICU cases in this study (n = 69) and to avoid overfitting in the model, eight variables were chosen for multivariate analysis based on previous findings and clinical constraints. A two-sided α of<0·05 was regarded statistically significant. Statistical analyses were performed using R version 4.0.4 (2021–02-15).

3. Results

3.1. Demographic characteristics

Table 1 summarized the demographics and laboratory characteristics of COVID-19 patients and HS. The results showed that the sex and age of individuals infected with COVID-19 were significantly different ($P < 0.05$). Besides, Table 2 and Fig. 1 indicated the difference between demographics and laboratory findings in ICU and non-ICU patients. Some factors, such as hospital discharge (duration time of hospitalization), death rate, mean cell hemoglobin (MCH), lactate dehydrogenase (LDH), glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), smoking were significantly higher in ICU patients than in non-ICU patients ($P < 0.05$). Table 3 illustrated that some risk factors such as age, lung disease, CRP, IL-4, IL-6, IL-8, IL-10, vitamin D were significantly different in deceased patients than alive patients. Further details are displayed in Table 3. Furthermore, we used multivariate regression analysis to determine the impact of confounding factors, such as age and sex. The results of multivariate regression analysis revealed that age and sex had no significant effects on the obtained results ($P > 0.05$) Table 2.1 and Table 3.1.Fig. 2.

3.2. Dynamic changes in the CRP, IL-4, IL-6, IL-8, 1L-10, NE, and vitamin D levels in ICU versus non-ICU patients

The levels of CRP, IL-4, IL-6, IL-8, 1L-10, NE, and Vitamin D in COVID-19 patients and HS are depicted in Table 2 and Fig. 1. Also, the levels of mentioned markers as well as demographic and laboratory results in ICU and non-ICU patients, were illustrated in Table 2. According to Table 2, our results revealed that the CRP, IL-4, IL-6, IL-8, IL-10, and NE were significantly higher in ICU patients than non-ICU patients ($P < 0.05$). Further analyses point out that the levels of IL-4, IL-6, IL-8, IL-10, and NE on the day of admission to T2 (3 days after

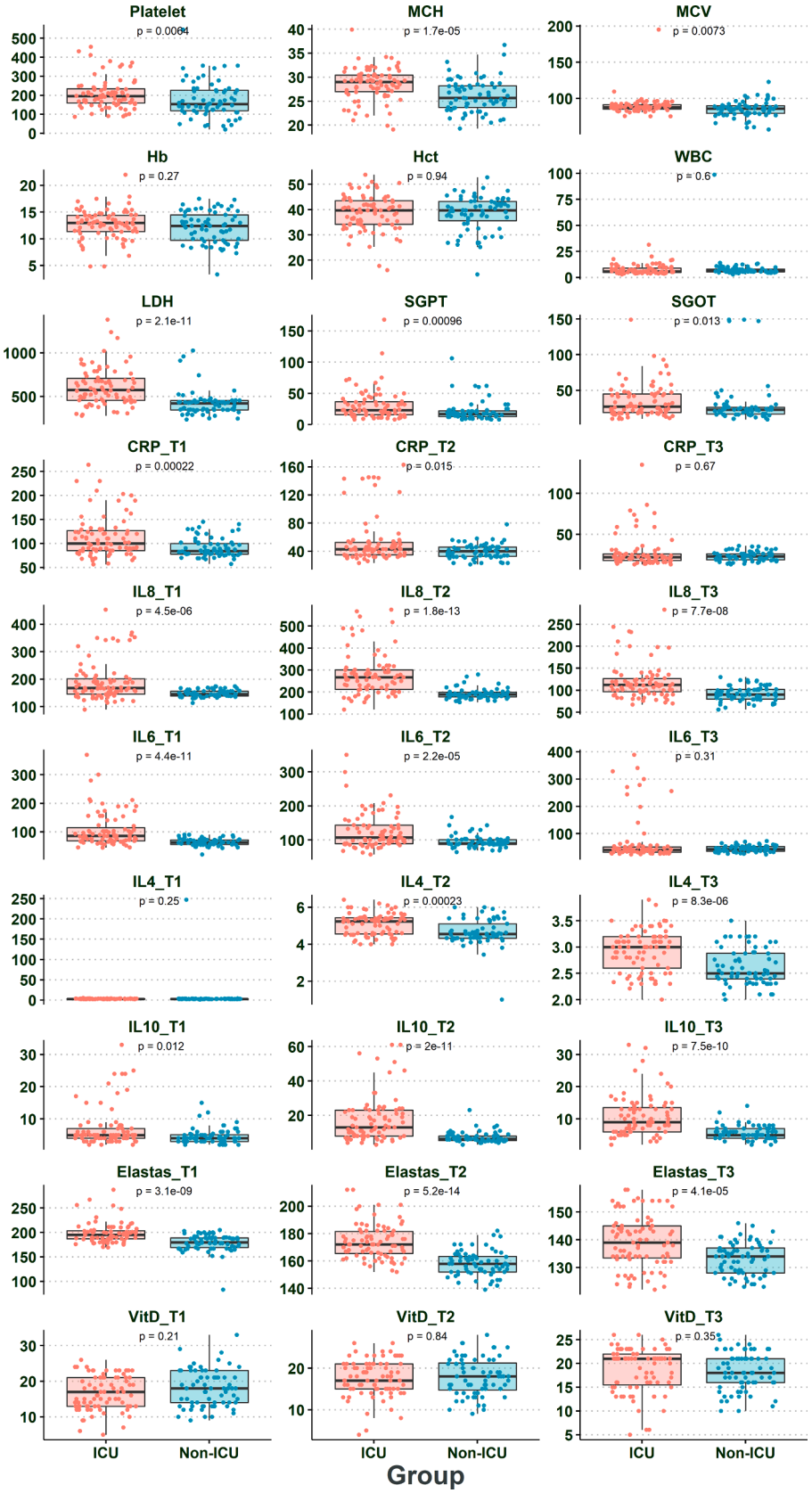

Fig. 1. The laboratory finding and dynamic changes in CRP levels, IL-4, IL-6, IL-8, IL-10, and vitamin D in ICU and non-ICU patients.

Table 2.1
Multivariate logistic regression results between ICU and non-ICU patients.

Characteristic	OR	95% CI	p-value
Age	1.00	0.98, 1.03	0.8
Sex			
Female	1.00		
Male	0.47	0.18, 1.20	0.12
Cigarette			
Negative	1.00		
Positive	5.97	1.35, 36.3	0.029
CRP_T1	1.02	1.00, 1.04	0.12
IL8_T1	1.01	0.99, 1.04	0.4
IL6_T1	1.06	1.02, 1.10	0.002
IL10_T1	1.12	0.94, 1.35	0.2
Elastas_T1	1.09	1.05, 1.14	<0.001

admission) were gradually increased in both ICU and non-ICU patients ($P < 0.05$), and also these markers were gradually reduced from T2 to T3 (6 days after admission) ($P < 0.05$). Concurrently, the CRP levels from the day of admission to T3 were gradually diminished ($P < 0.05$). We did not found significant differences in terms of vitamin D levels in ICU patients when compared with non-ICU patients ($P > 0.05$). Further details are shown in Table 2.

3.3. Dynamic alterations in the levels of CRP, IL-4, IL-6, IL-8, 1L-10, NE, and vitamin D in deceased patients in comparison with alive patients

The levels of CRP, IL-4, IL-6, IL-8, 1L-10, NE, and vitamin D in deceased patients versus alive patients were shown in Table 3. As shown in Table 2, the concentrations of the above marker were significantly higher in deceased patients than alive patients ($P < 0.05$). However, the vitamin D levels were significantly lower in deceased patients than in alive patients in T1 to T3 ($P < 0.05$). Similar to results obtained from the comparison between ICU and non-ICU patients, the serum concentrations of IL-4, IL-6, IL-8, IL-10, and NE on T1 were gently increased to T2 and then declined from T2 to T3. Further details are demonstrated in Table 3.

3.4. The impact of smoking on the dynamic changes in CRP, IL-4, IL-6, IL-8, 1L-10, NE, and vitamin D levels in patients with COVID-19

The results indicated that the frequency of smoking history in ICU patients was significantly higher than in non-ICU patients ($P < 0.05$). Also, further analyses showed that smoking history did not show any effects on the changes of CRP levels, IL-4, IL-6, IL-8, 1L-10, NE in comparison with those who were not smoker ($P > 0.05$). Of note, the only factor that was affected by smoking was vitamin D ($P < 0.05$). Smoking history was significantly associated with cardiovascular diseases, discharge from the hospital, alcohol consumption, and addiction ($P < 0.05$). Further details are displayed in Table 4.

3.5. The correlation between NE levels and pro/anti-inflammatory markers in ICU and non-ICU patients

In order to determine the correlation between serum NE levels and the levels of pro/anti-inflammatory markers, we used the matrix model of relationship. The results showed that on the day of admission (T1), the patterns of association between NE and pro/anti-inflammatory markers from low to high orders were IL-4 < IL-10 < IL-6 < IL-8 < CRP. Also, the correlation patterns on T2 and T3 from low to high orders were indicated as follows T2: IL-4 < CRP < IL-10 < IL-8 < IL-6, and T3: IL-4 < IL-6 < CRP < IL-8 < IL10, respectively (Fig. 3).Fig. 4.

3.6. The value of NE compared to other markers in prognosis of patients with COVID-19

The ROC analysis demonstrated that NE and other indices, such IL-4,

Table 3
Demographic and clinical parameters of deceased patients versus alive patients.

Variable	N	Overall, N = 152[1]	Death		p-value[2]
			Negative, N = 142[1]	Positive, N = 10[1]	
Group	152				0.002
ICU		83 (55%)	73 (51%)	10 (100%)	
Non-ICU		69 (45%)	69 (49%)	0 (0%)	
Sex	152				>0.9
Female		64 (42%)	60 (42%)	4 (40%)	
Male		88 (58%)	82 (58%)	6 (60%)	
Age	152	56 (38, 68)	54 (37, 66)	77 (72, 83)	<0.001
Platelet	152	175 (140, 232)	178 (142, 234)	160 (126, 174)	0.2
MCH	152	27.9 (25.0, 29.9)	27.8 (24.9, 29.9)	28.4 (27.3, 29.3)	0.5
MCV	152	87 (83, 91)	87 (83, 91)	87 (84, 88)	0.6
Hb	152	12.75 (10.60, 14.43)	12.70 (10.40, 14.30)	14.75 (11.88, 15.88)	0.068
Hct	152	40 (34, 43)	40 (34, 43)	41 (35, 48)	0.4
WBC	152	6.30 (5.10, 8.65)	6.30 (5.30, 8.75)	6.05 (4.85, 8.38)	0.7
LDH	152	453 (374, 650)	453 (368, 622)	586 (450, 656)	0.15
SGPT	152	19 (15, 32)	19 (15, 32)	18 (15, 30)	>0.9
SGOT	152	24 (18, 41)	24 (18, 41)	24 (22, 58)	0.7
Cigarette	152				>0.9
Negative		132 (87%)	123 (87%)	9 (90%)	
Positive		20 (13%)	19 (13%)	1 (10%)	
Opium	152				>0.9
Negative		148 (97%)	138 (97%)	10 (100%)	
Positive		4 (2.6%)	4 (2.8%)	0 (0%)	
Alcohol	152				0.4
Negative		145 (95%)	136 (96%)	9 (90%)	
Positive		7 (4.6%)	6 (4.2%)	1 (10%)	
Kidney. failure	152				0.6
Negative		131 (86%)	123 (87%)	8 (80%)	
Positive		21 (14%)	19 (13%)	2 (20%)	
Dialysis	152				0.2
Negative		141 (93%)	133 (94%)	8 (80%)	
Positive		11 (7.2%)	9 (6.3%)	2 (20%)	
Lung. disease	152				0.033
Negative		131 (86%)	125 (88%)	6 (60%)	
Positive		21 (14%)	17 (12%)	4 (40%)	
Heart. disease	152				0.060
Negative		127 (84%)	121 (85%)	6 (60%)	
Positive		25 (16%)	21 (15%)	4 (40%)	
Discharg	152	7 (5, 8)	7 (6, 8)	0 (0, 0)	<0.001
CRP_T1	152	90 (79, 120)	89 (79, 110)	195 (189, 208)	<0.001
CRP_T2	152	43 (34, 48)	42 (34, 46)	143 (126, 145)	<0.001
CRP_T3	152	22 (19, 26)	22 (18, 26)	70 (59, 78)	<0.001
IL8_T1	152	155 (140, 174)	154 (140, 168)	349 (342, 359)	<0.001
IL8_T2	152	204 (184, 276)	200 (182, 266)	489 (465, 536)	<0.001
IL8_T3	152	101 (87, 116)	100 (86, 113)	206 (197, 234)	<0.001
IL6_T1	152	72 (59, 90)	70 (59, 86)	205 (190, 264)	<0.001
IL6_T2	151	98 (87, 123)	97 (87, 111)	204 (191, 252)	<0.001
IL6_T3	151	43 (33, 53)	41 (33, 49)	274 (247, 321)	<0.001
IL4_T1	152	3.00 (2.50, 3.20)	3.00 (2.50, 3.20)	2.65 (2.50, 3.08)	0.5
IL4_T2	152	4.71 (4.43, 5.40)	4.69 (4.41, 5.40)	5.12 (4.52, 5.42)	0.6
IL4_T3	151	2.80 (2.44, 3.10)	2.77 (2.44, 3.10)	3.10 (3.02, 3.10)	0.018
IL10_T1	152	5.0 (3.7, 6.0)	4.4 (3.0, 5.8)	22.5 (9.0, 24.0)	<0.001

(continued on next page)

Table 3 (*continued*)

Variable	N	Overall, N = 152[1]	Death		p-value[2]
			Negative, N = 142[1]	Positive, N = 10[1]	
IL10_T2	151	8 (6, 14)	8 (6, 13)	48 (29, 55)	<0.001
IL10_T3	152	7 (5, 10)	6 (5, 9)	22 (12, 27)	<0.001
Elastas_T1	152	189 (178, 199)	189 (178, 198)	235 (190, 256)	0.005
Elastas_T2	151	165 (158, 176)	165 (158, 172)	190 (175, 201)	0.001
Elastas_T3	151	136 (130, 142)	135 (130, 142)	140 (130, 150)	0.3
VitD_T1	152	17 (13, 21)	18 (14, 21)	10 (8, 12)	<0.001
VitD_T2	151	18 (15, 21)	18 (15, 21)	12 (8, 15)	<0.001
VitD_T3	151	20 (16, 22)	20 (16, 22)	14 (7, 16)	0.002
Ct_value	151	29 (24, 33)	29 (24, 33)	24 (24, 34)	0.7

[1] n (%); Median (IQR)
[2] Fisher's exact test; Wilcoxon rank sum test

Table 3.1
Multivariate logistic regression results between deceased and alive patients.

Characteristic	OR	95% CI	p-value
Age	1.00	0.98, 1.03	0.7
Sex			
Female	1.00		
Male	0.42	0.18, 0.95	0.040
Lung. Disease			
Negative	1.00		
Positive	0.71	0.19, 2.74	0.6
CRP_T1	1.02	1.01, 1.04	0.003

IL-6, IL-8, IL-10, and CRP were significantly different between COVID-19 cases and HS. Also, according to the ROC curve analysis, area under the curve (AUC) of NE and other markers, including CRP, IL-4, IL-6, IL-8, and IL-10, on the first day between ICU and non-ICU patients were 0.7795, 0.6745, 0.5542, 0.8109, 0.7163, and 0.6174, respectively. Overall, the ROC results indicated that NE alone or in combination with IL-6 and IL-8 might be suitable candidates for monitoring the disease activity in patients with COVID-19.

4. Discussion

Neutrophils are frontier cells of innate immunity in protecting against pathogens and throughout the acute phase of inflammatory responses [31]. In current years, experimental data have recommended that there may be different subtypes of neutrophils with distinct functions in infection, cancer, and autoimmunity [32].

The participation of neutrophils in defense against various bacterial and fungal diseases is well established. However, the function of neutrophils in responses to viruses (which replicate intracellularly) has been less investigated [23]. Recently, accumulating evidence points out the critical role of neutrophils in the pathogenesis of COVID-19, especially in those with acute disease phases [31,33–35]. Therefore, this study investigated the possible correlation between NE and inflammatory markers to elucidate whether a dynamic change in NE levels correlates with inflammation and patient outcomes.

Our results noted that the levels of NE were significantly higher in patients with COVID-19 than HS. Also, our study findings displayed that NE levels in ICU patients were significantly elevated than in non-ICU patients. Our results were in line with a recent study conducted by Louis Guéant et al. [30] who revealed that the NE levels were

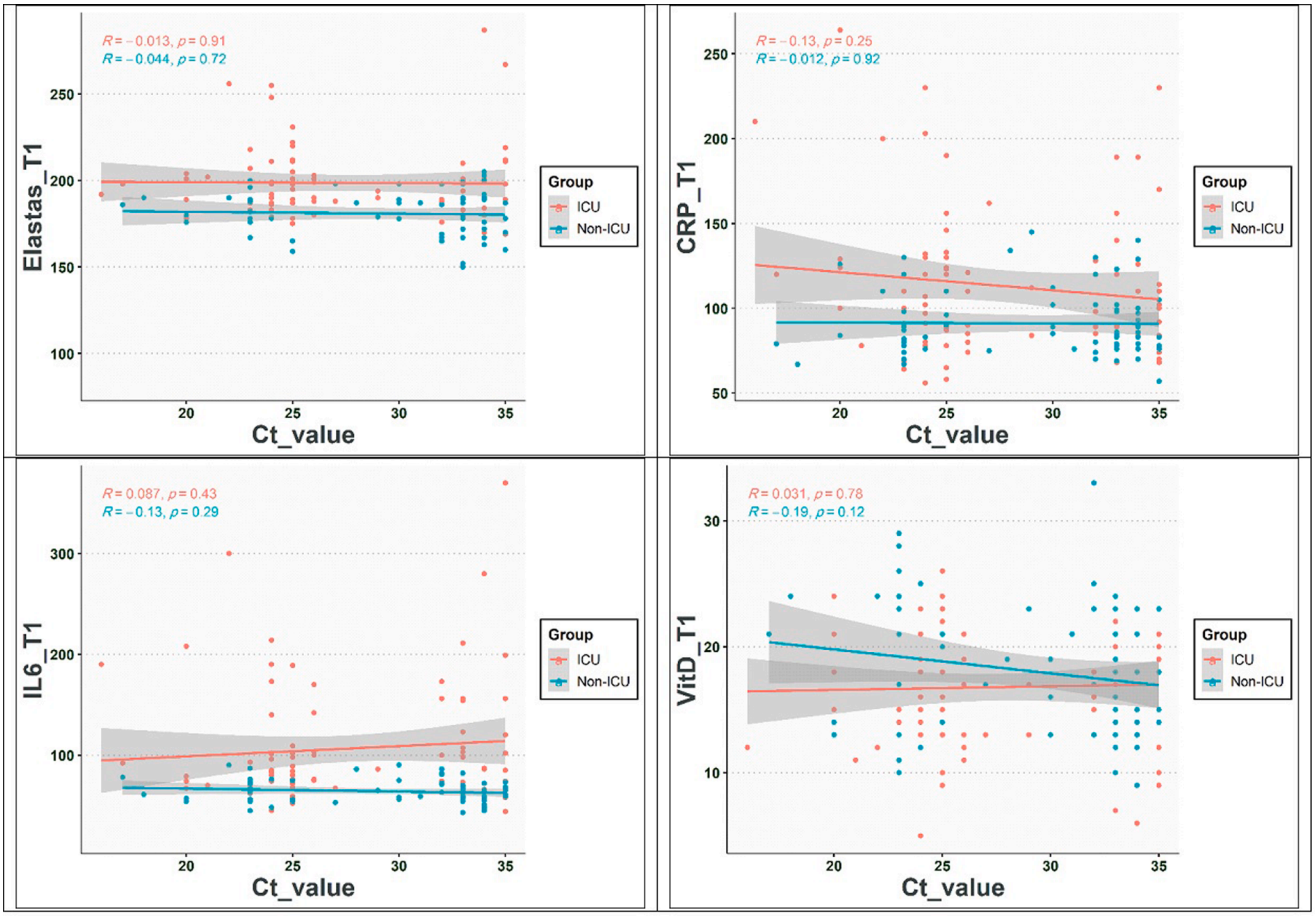

Fig. 2. The correlation between NE, IL-6, CRP, and vitamin D with CT value.

Table 4
Demographic and laboratory findings among smoker and non-smoker patients.

Variable	N	Overall, N = 152[1]	Cigarette		p-value[2]
			Negative, N = 132[1]	Positive, N = 20[1]	
Group	152				0.014
ICU		83 (55%)	67 (51%)	16 (80%)	
Non-ICU		69 (45%)	65 (49%)	4 (20%)	
Sex	152				0.8
Female		64 (42%)	55 (42%)	9 (45%)	
Male		88 (58%)	77 (58%)	11 (55%)	
Age	152	56 (38, 68)	55 (37, 67)	62 (47, 69)	0.2
Platelet	152	175 (140, 232)	174 (138, 228)	202 (174, 250)	0.072
MCH	152	27.9 (25.0, 29.9)	27.8 (24.8, 29.7)	27.9 (26.3, 30.2)	0.7
MCV	152	87 (83, 91)	87 (83, 91)	88 (84, 96)	0.3
Hb	152	12.75 (10.60, 14.43)	12.80 (10.60, 14.43)	12.55 (10.88, 14.25)	>0.9
Hct	152	40 (34, 43)	40 (35, 43)	37 (33, 45)	0.5
WBC	152	6.30 (5.10, 8.65)	6.15 (5.10, 8.53)	6.95 (5.23, 10.12)	0.4
LDH	152	453 (374, 650)	453 (374, 594)	626 (412, 733)	0.14
SGPT	152	19 (15, 32)	19 (15, 32)	22 (17, 32)	0.3
SGOT	152	24 (18, 41)	24 (18, 41)	22 (18, 34)	0.8
Opium	152				<0.001
Negative		148 (97%)	132 (100%)	16 (80%)	
Positive		4 (2.6%)	0 (0%)	4 (20%)	
Alcohol	152				0.049
Negative		145 (95%)	128 (97%)	17 (85%)	
Positive		7 (4.6%)	4 (3.0%)	3 (15%)	
Kidney. failure	152				>0.9
Negative		131 (86%)	113 (86%)	18 (90%)	
Positive		21 (14%)	19 (14%)	2 (10%)	
Dialysis	152				>0.9
Negative		141 (93%)	122 (92%)	19 (95%)	
Positive		11 (7.2%)	10 (7.6%)	1 (5.0%)	
Lung. disease	152				0.2
Negative		131 (86%)	116 (88%)	15 (75%)	
Positive		21 (14%)	16 (12%)	5 (25%)	
Heart. disease	152				0.025
Negative		127 (84%)	114 (86%)	13 (65%)	
Positive		25 (16%)	18 (14%)	7 (35%)	
Discharg	152	7 (5, 8)	6 (5, 8)	8 (6, 9)	0.023
Death	152				>0.9
Negative		142 (93%)	123 (93%)	19 (95%)	
Positive		10 (6.6%)	9 (6.8%)	1 (5.0%)	
CRP_T1	152	90 (79, 120)	90 (79, 120)	100 (87, 121)	0.2
CRP_T2	152	43 (34, 48)	43 (34, 48)	42 (33, 51)	0.9
CRP_T3	152	22 (19, 26)	22 (19, 26)	22 (17, 26)	0.6
IL8_T1	152	155 (140, 174)	156 (140, 172)	154 (140, 186)	>0.9
IL8_T2	152	204 (184, 276)	200 (184, 278)	226 (194, 270)	0.4
IL8_T3	152	101 (87, 116)	100 (87, 114)	110 (90, 126)	0.2
IL6_T1	152	72 (59, 90)	72 (59, 89)	72 (62, 94)	0.6
IL6_T2	151	98 (87, 123)	98 (87, 122)	100 (87, 126)	0.8
IL6_T3	151	43 (33, 53)	43 (34, 53)	37 (30, 51)	0.3
IL4_T1	152	3.00 (2.50, 3.20)	2.94 (2.49, 3.20)	3.05 (2.50, 3.25)	0.4
IL4_T2	152	4.71 (4.43, 5.40)	4.71 (4.40, 5.40)	4.92 (4.50, 5.40)	0.8
IL4_T3	151	2.80 (2.44, 3.10)	2.77 (2.44, 3.10)	3.05 (2.70, 3.24)	0.060
IL10_T1	152	5.0 (3.7, 6.0)	5.0 (4.0, 6.0)	4.5 (3.0, 5.2)	0.7
IL10_T2	151	8 (6, 14)	8 (6, 16)	8 (6, 11)	0.5
IL10_T3	152	7 (5, 10)	7 (5, 11)	6 (5, 7)	0.2
Elastas_T1	152	189 (178, 199)	188 (178, 198)	198 (186, 206)	0.074
Elastas_T2	151	165 (158, 176)	165 (158, 174)	170 (160, 183)	0.2
Elastas_T3	151	136 (130, 142)	135 (129, 142)	138 (134, 143)	0.3
VitD_T1	152	17 (13, 21)	17 (13, 21)	21 (17, 23)	0.035
VitD_T2	151	18 (15, 21)	17 (14, 21)	20 (17, 21)	0.082
VitD_T3	151	20 (16, 22)	19 (15, 21)	21 (18, 23)	0.026
Ct_value	151	29 (24, 33)	29 (24, 33)	25 (24, 33)	0.5

[1] n (%); Median (IQR)
[2] Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test

significantly elevated in COVID-19 patients. The recent evidence has represented the pathogenic role of NE in respiratory viral infections [36] and lung injury [27,37,38]. NE can lead to lung damage; however, this protease has not evolved to cause lung tissue injury; the elastin-rich connective tissue framework of the lungs seems to be especially sensitive to the function of elastolytic proteases. Assuming that NE most likely represents a function in the immigration of neutrophils toward a site of inflammation and degeneration of proteins from attacking organisms or other inflammatory response results, it is the performance of inhibitors of this protease to preserve normal tissues from its impacts [29]. These results may reflect the event in which the excessive activation of neutrophils, followed by the elevated levels of NE which can result in lung injury via serine protease effects.

The axial role of cytokine storm in the pathogenesis of COVID-19 has profoundly been appreciated [39–42]. Our data show that the levels of inflammatory indices, such as CRP, IL-6, and IL-8, were significantly enhanced in cases with COVID-19 than in HS. Additional analyses revealed that the serum concentrations of these inflammatory markers in ICU patients was significantly higher than in non-ICU patients. We have also showed that the levels of IL-6 and IL-8 were gradually increased from day 0 to day 3 in both ICU and non-ICU patients. However, the CRP levels were gently reduced from day 0 to day 6 in both groups of patients. Our finding confirms previous investigations demonstrating that the IL-6 [43–50], IL-8 [51–54], and CRP [55,56] were significantly elevated in patients with COVID-19. Besides, our results revealed that the concentrations of IL-4 and IL-10 were significantly elevated in cases with COVID-19 than in HS.

Additional analyses indicated that the levels of IL-4 and IL10 were significantly higher in ICU patients than non-ICU patients. Our findings were consistent with several studies that showed the levels of IL-4 [57] and IL-10 [43,58–60] elevated in patients with COVID-19. Recently, in infected alveolar macrophages, it has been appreciated that IL-6 and IL-8 are generated at the same time of NF-κB activation through mechanisms that plausibly involve Bruton tyrosine kinase (BTK). This is represented by the encouraging clinical outcomes provided by the BTK inhibitor acalabrutinib in critical COVID-19 patients [61]. IL-8 acts as neutrophil-activating chemokine when bound to CXCR2, which is a essential chemokine receptor of neutrophils [62]. Hence, a notable rise in NE in critical COVID-19 cases may be correlated to the activation of neutrophils through the IL-8/CXCR2 pathways [63].

The vast majority of observational research has verified a low vitamin D state associated with the occurrence of the upper respiratory viral infections [64–67]. Our results revealed that Vit D levels were significantly lower in COVID-19 cases than in HS. Additionally, there was no statistically significant difference in serum vitamin D levels between ICU and non-ICU patients. However, serum vitamin D levels were significantly lower in deceased patients than in alive patients. Our findings, in agreement with similar studies, indicate that COVID-19 patients had lower Vit D levels [67–70].

Finally, the ROC curve report shows that AUC from high to low is: IL-6 > NE > IL-8 > CRP > IL-10 > IL-4. Similarly, matrix correlation

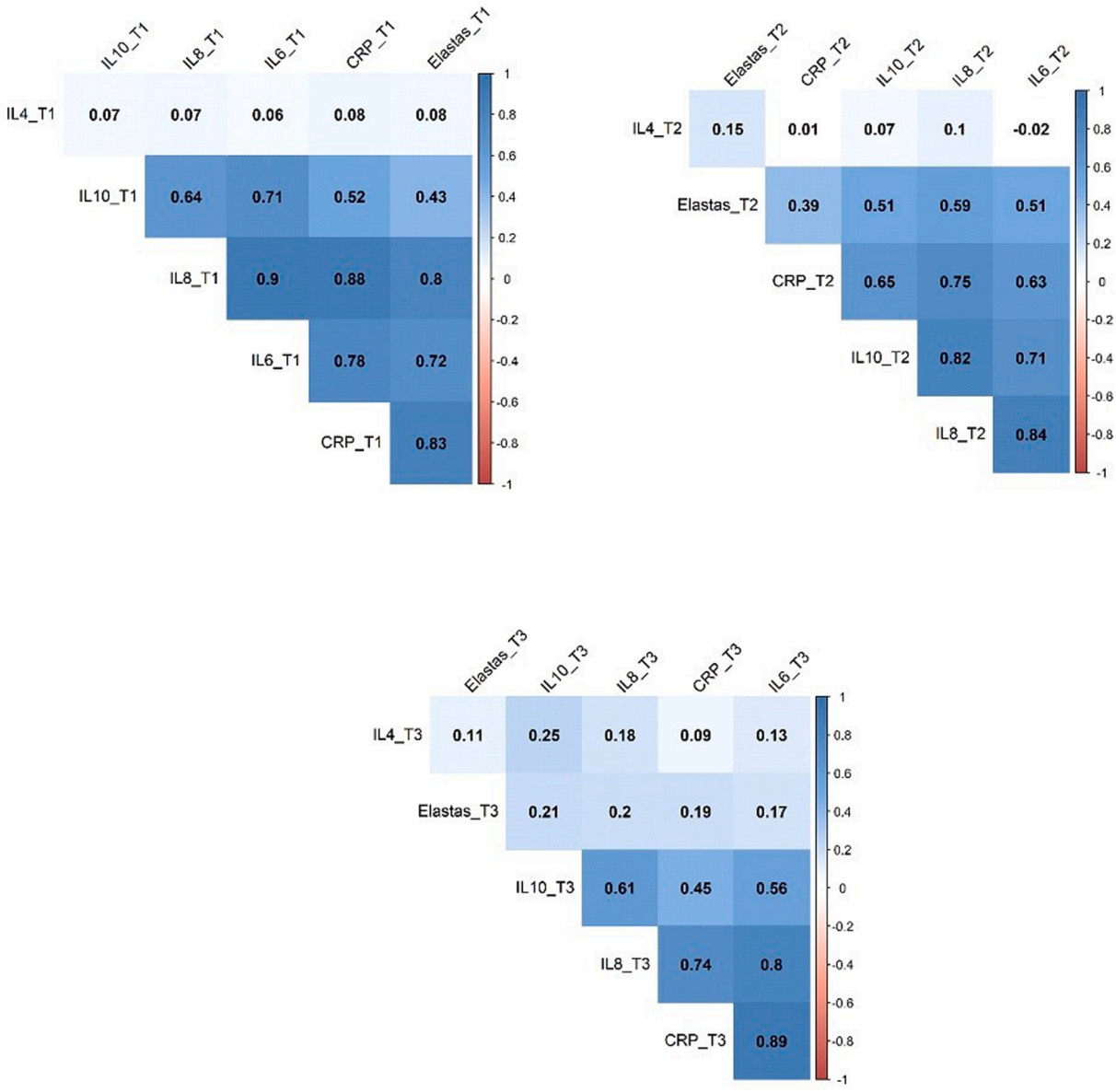

Fig. 3. The matrix correlation between NE and pro/anti-inflammatory markers.

showed the NE has a high correlation with CRP, IL-6, and IL-8. Overall, these findings implying that NE, along with IL-6 and IL-8, is a reliable indicator in differentiating severe SARS-CoV2 infection (ICU patients) cases from moderate ones (non-ICU patients).

5. Conclusion

Overall, we provided further significant supports for the pathogenic role of NE in COVID-19. Also, we have addressed a possible link between NE dynamic changes with inflammatory cytokines, implying that this enzyme alone or in combination with other markers, such as IL-6 and IL-8, could be employed for monitoring the disease activity. Ultimately, the elucidation of the interaction between NE and inflammatory cytokines may improve our knowledge to shed light on this enzyme's role in health and disease state. We have previously mentioned that NE is a serine proteinase that can damage lung tissue when the balance between protease and anti-proteases is disturbed, so in the case of COVID-19, the application of NE inhibitors may open up a therapeutic window for the treatment of patients with COVID-19.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

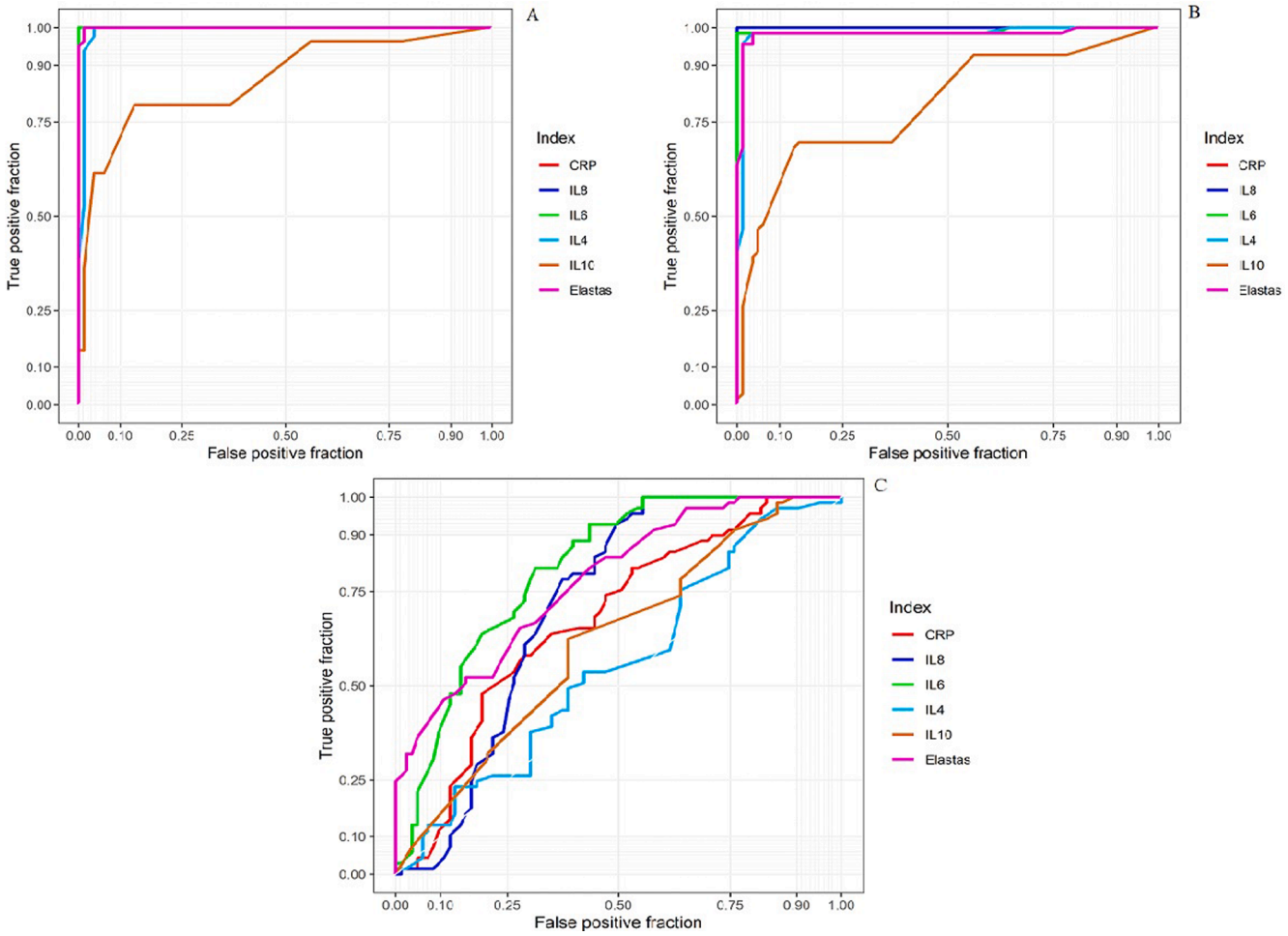

Fig. 4. ROC curve analysis between, A: Healthy people vs. ICU patients, B: Healthy people vs. Non-ICU patients, and C: Non-ICU patients vs. ICU patients. AUC: Area under the curve.

Acknowledgments

On behalf of the authors, we sincerely thank patients, healthy subjects, and clinicians (Firouzgar Hospital, Tehran, Iran) for their collaborations.

Ethical approval

The Iran University of Medical Sciences Ethics Review Board approved this study (No. IR.IUMS.FMD.REC.1399.624(.

References

[1] P. Goodarzi, F. Mahdavi, R. Mirzaei, H. Hasanvand, M. Sholeh, F. Zamani, M. Sohrabi, A. Tabibzadeh, A.S. Jeda, M.H.K. Niya, Coronavirus disease, Coronavirus disease 2019 (COVID-19): Immunological approaches and emerging pharmacologic treatments, Int Immunopharmacol (2020), 106885.

[2] J.M. Sanders, M.L. Monogue, T.Z. Jodlowski, J.B. Cutrell, Pharmacologic treatments for coronavirus disease 2019 (COVID-19): a review, Jama 323 (18) (2020) 1824–1836.

[3] H. Keyvani, H. Zahednasab, M. Sholeh, R. Mirzaei, M. Esghaei, S. Karampoor, Gender Preponderance Might be Associated with the Severity of COVID-19 Infection, J Clin Cell Immunol 11 (2020) 598.

[4] R. Mirzaei, R. Mohammadzadeh, F. Mahdavi, F. Badrzadeh, S. Kazemi, M. Ebrahimi, F. Soltani, S. Kazemi, A.S. Jeda, M. Darvishmotevalli, Overview of the current promising approaches for the development of an effective severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine, Int Immunopharmacol 106928 (2020).

[5] H. Li, S.-M. Liu, X.-H. Yu, S.-L. Tang, C.-K. Tang, Coronavirus disease 2019 (COVID-19): current status and future perspectives, International journal of antimicrobial agents 55 (5) (2020), 105951.

[6] R. Mirzaei, S. Karampoor, M. Sholeh, P. Moradi, R. Ranjbar, F. Ghasemi, A contemporary review on pathogenesis and immunity of COVID-19 infection, Molecular biology reports 47 (7) (2020) 5365–5376.

[7] C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, The lancet 395 (10223) (2020) 497–506.

[8] P. Zhou, X.-L. Yang, X.-G. Wang, B. Hu, L. Zhang, W. Zhang, H.-R. Si, Y. Zhu, B. Li, C.-L. Huang, A pneumonia outbreak associated with a new coronavirus of probable bat origin, nature 579 (7798) (2020) 270–273.

[9] G. Grasselli, A. Zangrillo, A. Zanella, M. Antonelli, L. Cabrini, A. Castelli, D. Cereda, A. Coluccello, G. Foti, R. Fumagalli, Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy Region, Italy, Jama 323 (16) (2020) 1574–1581.

[10] A.K. Azkur, M. Akdis, D. Azkur, M. Sokolowska, W. van de Veen, M.C. Brüggen, L. O'Mahony, Y. Gao, K. Nadeau, C.A. Akdis, Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19, Allergy 75 (7) (2020) 1564–1581.

[11] D. McGonagle, K. Sharif, A. O'Regan, C. Bridgewood, The role of cytokines including interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease, Autoimmunity reviews 19 (6) (2020), 102537.

[12] P. Mo, Y. Xing, Y. Xiao, L. Deng, Q. Zhao, H. Wang, Y. Xiong, Z. Cheng, S. Gao, K. Liang, Clinical characteristics of refractory COVID-19 pneumonia in Wuhan, China, Clinical Infectious Diseases (2020).

[13] V. Papayannopoulos, Neutrophil extracellular traps in immunity and disease, Nature Reviews Immunology 18 (2) (2018) 134.

[14] J. Liu, Z. Pang, G. Wang, X. Guan, K. Fang, Z. Wang, F. Wang, Advanced role of neutrophils in common respiratory diseases, Journal of immunology research 2017 (2017).

[15] J.V. Camp, C.B. Jonsson, A role for neutrophils in viral respiratory disease, Frontiers in immunology 8 (2017) 550.

[16] S.-C. Yang, Y.-F. Tsai, Y.-L. Pan, T.-L. Hwang, Understanding the role of neutrophils in acute respiratory distress syndrome, Biomedical Journal (2020).

[17] B.J. Barnes, J.M. Adrover, A. Baxter-Stoltzfus, A. Borczuk, J. Cools-Lartigue, J. M. Crawford, J. Daßler-Plenker, P. Guerci, C. Huynh, J.S. Knight, Targeting potential drivers of COVID-19: Neutrophil extracellular traps, Journal of Experimental Medicine 217 (6) (2020).

[18] Y. Zuo, Y. Srilakshmi, S. Hui, G. Kelsey, Melanie Zet al, Neutrophil extracellular traps in COVID-19, JCI Insight 5 (11) (2020), e138999.
[19] J.L. Guéant, J. Fromonot, R.M. Guéant-Rodriguez, P. Lacolley, R. Guieu, V. Regnault, Blood myeloperoxidase-DNA, a biomarker of early response to SARS-CoV-2 infection? Allergy (2020).
[20] N. Borregaard, Development of neutrophil granule diversity, Annals of the New York Academy of Sciences 832 (1) (1997) 62–68.
[21] M. Emboriadou, M. Hatzistilianou, C. Magnisali, A. Sakelaropoulou, M. Exintari, P. Conti, V. Aivazis, Human neutrophil elastase in RSV bronchiolitis, Annals of Clinical & Laboratory Science 37 (1) (2007) 79–84.
[22] M. Abu-Harb, F. Bell, A. Finn, W. Rao, L. Nixon, D. Shale, M. Everard, IL-8 and neutrophil elastase levels in the respiratory tract of infants with RSV bronchiolitis, European Respiratory Journal 14 (1) (1999) 139–143.
[23] C. Johansson, F.C. Kirsebom, Neutrophils in respiratory viral infections, Mucosal Immunology (2021) 1–13.
[24] N. Song, W. Wang, Y. Wang, Y. Guan, S. Xu, M.-Y. Guo, Hydrogen sulfide of air induces macrophage extracellular traps to aggravate inflammatory injury via the regulation of miR-15b-5p on MAPK and insulin signals in trachea of chickens, Science of The Total Environment 771 (2021), 145407.
[25] H. Jing, Q. Zhang, X.-J. Gao, Excessive lithium of water induced a toxic effect on kidney via oxidative damage and inflammation in carp, Aquaculture 535 (2021), 736282.
[26] H. Jing, S. Wang, Y. Wang, N. Shen, X.-J. Gao, Environmental contaminant ammonia triggers epithelial-to-mesenchymal transition-mediated jejunal fibrosis with the disassembly of epithelial cell-cell contacts in chicken, Science of The Total Environment 726 (2020), 138686.
[27] E. Polverino, E. Rosales-Mayor, G.E. Dale, K. Dembowsky, A. Torres, The role of neutrophil elastase inhibitors in lung diseases, Chest 152 (2) (2017) 249–262.
[28] I.E. Galani, E. Andreakos, Neutrophils in viral infections: current concepts and caveats, Journal of leukocyte biology 98 (4) (2015) 557–564.
[29] R.A. Sandhaus, G. Turino, Neutrophil elastase-mediated lung disease, COPD: Journal of Chronic Obstructive Pulmonary Disease 10 (sup1) (2013) 60–63.
[30] J.L. Gueant, R.M. Rodriguez-Gueant, J. Fromonot, A. Oussalah, H. Louis, C. Chery, M. Gette, S. Gleye, J. Callet, J. Raso, Elastase and Exacerbation of Neutrophil Innate Immunity are Involved in Multi-Visceral Manifestations of COVID-19, Authorea Preprints (2020).
[31] N. Reusch, E. De Domenico, L. Bonaguro, J. Schulte-Schrepping, K. Baßler, J. L. Schultze, A.C. Aschenbrenner, Neutrophils in COVID-19, Frontiers in Immunology 12 (2021).
[32] L.G. Ng, R. Ostuni, A. Hidalgo, Heterogeneity of neutrophils, Nature Reviews Immunology 19 (4) (2019) 255–265.
[33] D. Dennison, M. Al Khabori, S. Al Mamari, A. Aurelio, H. Al Hinai, K. Al Maamari, J. Alshekaili, G. Al Khadouri, Circulating activated neutrophils in COVID-19: An independent predictor for mechanical ventilation and death, International Journal of Infectious Diseases 106 (2021) 155–159.
[34] A.C. Aschenbrenner, M. Mouktaroudi, B. Kraemer, M. Oestreich, N. Antonakos, M. Nuesch-Germano, K. Gkizeli, L. Bonaguro, N. Reusch, K. Baßler, Disease severity-specific neutrophil signatures in blood transcriptomes stratify COVID-19 patients, Genome Medicine 13 (1) (2021) 1–25.
[35] M.L. Meizlish, A.B. Pine, J.D. Bishai, G. Goshua, E.R. Nadelmann, M. Simonov, C.-H. Chang, H. Zhang, M. Shallow, P. Bahel, A neutrophil activation signature predicts critical illness and mortality in COVID-19, Blood advances 5 (5) (2021) 1164–1177.
[36] B.M. Tang, M. Shojaei, S. Teoh, A. Meyers, J. Ho, T.B. Ball, Y. Keynan, A. Pisipati, A. Kumar, D.P. Eisen, Neutrophils-related host factors associated with severe disease and fatality in patients with influenza infection, Nature communications 10 (1) (2019) 1–13.
[37] K. Kawabata, T. Hagio, S. Matsuoka, The role of neutrophil elastase in acute lung injury, European journal of pharmacology 451 (1) (2002) 1–10.
[38] R. Boxio, J. Wartelle, B. Nawrocki-Raby, B. Lagrange, L. Malleret, T. Hirche, C. Taggart, Y. Pacheco, G. Devouassoux, A. Bentaher, Neutrophil elastase cleaves epithelial cadherin in acutely injured lung epithelium, Respiratory research 17 (1) (2016) 1–15.
[39] R.Q. Cron, COVID-19 cytokine storm: targeting the appropriate cytokine, The Lancet, Rheumatology 3 (4) (2021) e236–e237.
[40] L.F. García, Immune response, inflammation, and the clinical spectrum of COVID-19, Frontiers in immunology 11 (2020) 1441.
[41] F. Coperchini, L. Chiovato, L. Croce, F. Magri, M. Rotondi, The cytokine storm in COVID-19: An overview of the involvement of the chemokine/chemokine-receptor system, Cytokine & growth factor reviews 53 (2020) 25–32.
[42] D. Ragab, H. Salah Eldin, M. Taeimah, R. Khattab, R. Salem, The COVID-19 cytokine storm; what we know so far, Frontiers in immunology 11 (2020) 1446.
[43] S.K. Dhar, K. Vishnupriyan, S. Damodar, S. Gujar, M. Das, IL-6 and IL-10 as predictors of disease severity in COVID-19 patients: results from meta-analysis and regression, Heliyon 7 (2) (2021), e06155.
[44] D.D. Fraser, G. Cepinskas, M. Slessarev, C. Martin, M. Daley, M.R. Miller, D. B. O'Gorman, S.E. Gill, E.K. Patterson, C.C. Dos Santos, Inflammation profiling of critically ill coronavirus disease 2019 patients, Critical care explorations 2 (6) (2020).
[45] E. Nasonov, M. Samsonov, The role of Interleukin 6 inhibitors in therapy of severe COVID-19, Biomedicine & Pharmacotherapy 110698 (2020).
[46] G. Cavalli, A. Larcher, A. Tomelleri, C. Campochiaro, E. Della-Torre, G. De Luca, N. Farina, N. Boffini, A. Ruggeri, A. Poli, Interleukin-1 and interleukin-6 inhibition compared with standard management in patients with COVID-19 and hyperinflammation: a cohort study, The Lancet Rheumatology 3 (4) (2021) e253–e261.
[47] J. Zhang, Y. Hao, W. Ou, F. Ming, G. Liang, Y. Qian, Q. Cai, S. Dong, S. Hu, W. Wang, Serum interleukin-6 is an indicator for severity in 901 patients with SARS-CoV-2 infection: a cohort study, Journal of translational medicine 18 (1) (2020) 1–8.
[48] J.J. Guirao, C.M. Cabrera, N. Jiménez, L. Rincón, J.M. Urra, High serum IL-6 values increase the risk of mortality and the severity of pneumonia in patients diagnosed with COVID-19, Molecular Immunology 128 (2020) 64–68.
[49] H. Sun, P. Guo, L. Zhang, F. Wang, Serum Interleukin-6 Concentrations and the Severity of COVID-19 Pneumonia: A Retrospective Study at a Single Center in Bengbu City, Anhui Province, China, in January and February 2020, Medical Science Monitor: International Medical Journal of Experimental and Clinical Research 26 (2020) e926941–e926951.
[50] S. Karampoor, H. Zahednasab, M. Farahmand, R. Mirzaei, F. Zamani, A. Tabibzadeh, B. Bouzari, H. Ajdarkosh, M. Nikkhah, M.R. Hashemi, A possible pathogenic role of Syndecan-1 in the pathogenesis of coronavirus disease 2019 (COVID-19), Int Immunopharmacol 97 (2021) 107684.
[51] A. Ma, L. Zhang, X. Ye, J. Chen, J. Yu, L. Zhuang, C. Weng, F. Petersen, Z. Wang, X. Yu, High Levels of Circulating IL-8 and Soluble IL-2R Are Associated With Prolonged Illness in Patients With Severe COVID-19, Frontiers in immunology 12 (2021) 12.
[52] D. Darif, I. Hammi, A. Kihel, I.E.I. Saik, F. Guessous, K. Akarid, The pro-inflammatory cytokines in COVID-19 pathogenesis: What goes wrong? Microbial Pathogenesis 104799 (2021).
[53] H. Codina, I. Vieitez, A. Gutierrez-Valencia, V. Skouridou, C. Martínez, L. Patiño, M. Botero-Gallego, M. Trujillo-Rodríguez, A. Serna-Gallego, E. Muñoz-Muela, Elevated anti-SARS-CoV-2 antibodies and IL-6, IL-8, MIP-1β, early predictors of severe COVID-19, bioRxiv (2021).
[54] H. Li, J. Zhang, C. Fang, X. Zhao, B. Qian, Y. Sun, Y. Zhou, J. Hu, Y. Huang, Q. Ma, The prognostic value of IL-8 for the death of severe or critical patients with COVID-19, Medicine 100 (11) (2021).
[55] M. Ahnach, S. Zbiri, S. Nejjari, F. Ousti, C. Elkettani, C-reactive protein as an early predictor of COVID-19 severity, Journal of Medical Biochemistry 39 (4) (2020) 500.
[56] L. Wang, C-reactive protein levels in the early stage of COVID-19, Medecine et maladies infectieuses 50 (4) (2020) 332–334.
[57] C.B.V. de Paula, M.L.V. de Azevedo, S. Nagashima, A.P.C. Martins, M.A.S. Malaquias, A.F.R. dos Santos Miggiolaro, J.d.S.M. Júnior, G. Avelino, L.A.P. do Carmo, L.B. Carstens, IL-4/IL-13 remodeling pathway of COVID-19 lung injury, Scientific Reports 10(1) (2020) 1-8.
[58] H. Han, Q. Ma, C. Li, R. Liu, L. Zhao, W. Wang, P. Zhang, X. Liu, G. Gao, F. Liu, Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors, Emerging microbes & infections 9 (1) (2020) 1123–1130.
[59] L. Lu, H. Zhang, D.J. Dauphars, Y.-W. He, A Potential Role of Interleukin-10 in COVID-19 Pathogenesis, Trends in Immunology (2020).
[60] J. Li, L. Rong, R. Cui, J. Feng, Y. Jin, Y. Yu, X. Chen, R. Xu, Dynamic changes in serum IL-6, IL-8, and IL-10 are associated with the outcome of patients with severe COVID-19 in ICU, (2020).
[61] M. Roschewski, M.S. Lionakis, J.P. Sharman, J. Roswarski, A. Goy, M.A. Monticelli, M. Roshon, S.H. Wrzesinski, J.V. Desai, M.A. Zarakas, Inhibition of Bruton tyrosine kinase in patients with severe COVID-19, Science immunology 5 (48) (2020).
[62] M. Uddin, H. Watz, A. Malmgren, F. Pedersen, NETopathic inflammation in chronic obstructive pulmonary disease and severe asthma, Frontiers in immunology 10 (2019) 47.
[63] D. Athanasios, COVID-19 Hyperinflammation: What about Neutrophils? mSphere 5 (3) (2020).
[64] K. Bryson, A. Nash, M. Norval, Does vitamin D protect against respiratory viral infections? Epidemiology & Infection 142 (9) (2014) 1789–1801.
[65] M. Balla, G.P. Merugu, V.M. Konala, V. Sangani, H. Kondakindi, M. Pokal, V. Gayam, S. Adapa, S. Naramala, S.V. Malayala, Back to basics: review on vitamin D and respiratory viral infections including COVID-19, Journal of community hospital internal medicine perspectives 10 (6) (2020) 529–536.
[66] J. Charan, J.P. Goyal, D. Saxena, P. Yadav, Vitamin D for prevention of respiratory tract infections: A systematic review and meta-analysis, Journal of pharmacology & pharmacotherapeutics 3 (4) (2012) 300.
[67] A. Jain, R. Chaurasia, N.S. Sengar, M. Singh, S. Mahor, S. Narain, Analysis of vitamin D level among asymptomatic and critically ill COVID-19 patients and its correlation with inflammatory markers, Scientific reports 10 (1) (2020) 1–8.
[68] E. Merzon, D. Tworowski, A. Gorohovski, S. Vinker, A. Golan Cohen, I. Green, M. Frenkel-Morgenstern, Low plasma 25 (OH) vitamin D level is associated with increased risk of COVID-19 infection: an Israeli population-based study, The FEBS journal 287 (17) (2020) 3693–3702.
[69] G.E. Carpagnano, V. Di Lecce, V.N. Quaranta, A. Zito, E. Buonamico, E. Capozza, A. Palumbo, G. Di Gioia, V.N. Valerio, O. Resta, Vitamin D deficiency as a predictor of poor prognosis in patients with acute respiratory failure due to COVID-19, Journal of endocrinological investigation 44 (4) (2021) 765–771.
[70] G. Panagiotou, S.A. Tee, Y. Ihsan, W. Athar, G. Marchitelli, D. Kelly, C.S. Boot, N. Stock, J. Macfarlane, A.R. Martineau, Low serum 25-hydroxyvitamin D (25 [OH] D) levels in patients hospitalized with COVID-19 are associated with greater disease severity, Clinical endocrinology 93 (4) (2020) 508–511.