Factors predicting poor outcomes of patients treated with tocilizumab for COVID-19-associated pneumonia: A retrospective study

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Abstract. The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic is a significant global issue that has major implications for the healthcare system. The mortality rates associated with SARS-CoV-2 infection vary according to the geographical region and are associated with age, comorbidities and vaccination status. Organ damage is caused by the cytokine release syndrome, which plays a crucial role in the course of coronavirus disease 2019 (COVID-19) infection. Innate and adaptive immune system stimulation in patients with COVID-19 results in inappropriate cytokine release. The anti-IL-6 receptor antagonist, tocilizumab, is used in the treatment of connective tissue diseases. The present single-center retrospective study on patients with COVID-19 admitted to hospital between September, 2020 and April, 2022 aimed to identify predictors of mortality and other unfavorable outcomes in patients treated with tocilizumab for COVID-19-associated pneumonia. Demographics, vaccination status against SARS-CoV-2, the Charlson comorbidity index (CCI), laboratory data and chest X-ray scores were recorded upon admission. In total, 174 subjects (121 males; mean age, 62.43±13.47 years) fulfilling the inclusion criteria were included. Among the 174 participants, 58 (33.3%) were intubated. The mortality rate was 35.1%. The non-survivors were older, mostly females, and had a higher CCI score. At the evaluation upon admission, the survivors presented with higher levels of alanine transferase and gamma glutamyl-transferase and with a greater number of platelets (PLTs), while patients that were intubated were also older, mostly females, and had a higher CCI score ($P<0.05$). Age was identified as the only independent factor predicting mortality in the Cox proportional hazards multivariate regression analysis. By performing a sub-analysis regarding sex, it was revealed that the value of PLTs was an independent factor predicting intubation and 90-day mortality in male patients, and the lymphocyte count was the only factor associated with intubation in female patients. On the whole, the data of the present study may be used to identify patient subpopulations responding to treatment with tocilizumab in prospective clinical trials.

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Introduction

As of July 14, 2022, the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has caused 6,356,812 deaths globally (1). The mortality rates associated with SARS-CoV-2 infection vary according to the geographical region and are associated with age, comorbidities and vaccination status (2). Organ damage is considered to be caused by the cytokine release syndrome, which is crucial during the course of coronavirus disease 2019 (COVID-19) infection. Organ damage is

also caused by septic shock, thrombosis and oxidative stress (3). Excessive cytokine release in patients with COVID-19 is induced by the stimulation of the innate and adaptive immune systems. An unbalanced immune response and excessive inflammation are key pathogenic factors in COVID-19 (4).

Interleukin (IL)-6) is secreted by macrophages in response to specific microbial molecules known as pathogen-associated molecular patterns. These patterns bind to a key type of innate immune system detection molecules known as pattern recognition receptors, which include Toll-like receptors. These are found on the cell surface and in intracellular compartments, and they initiate intracellular signaling cascades, resulting in the release of inflammatory cytokines (5). IL-6 has been found to be implicated in severe SARS-CoV-2 infection (6). IL-6 levels of 80 pg/ml suggest an increased risk of respiratory failure and mortality, and immunomodulatory therapy is an area of urgent research (6).

Tocilizumab is an anti-IL-6 receptor antagonist that is used in the treatment of connective tissue disorders, such as rheumatoid arthritis, giant cell arteritis, polyarticular juvenile idiopathic arthritis and systemic juvenile idiopathic arthritis (7). This agent has exhibited efficacy against COVID-19 (8-12). The RECOVERY trial reported a decrease in the mortality rate from 35 to 31% when corticosteroids were used simultaneously in hospitalized patients with moderate, severe or critical COVID-19 infection, and with evidence of inflammation (8). Another study demonstrated that patients who were critically ill with COVID-19 who received tocilizumab or sarilumab presented with a mortality rate of 27% in the REMAP-CAP trial, compared to 36% in the control group receiving only standard care (9). Furthermore, another three meta-analyses (10-12) all agreed that tocilizumab should be used in the treatment of patients with severe COVID-19 infection. The first revealed a pooled mortality rate of 19% in the tocilizumab group (10), the second revealed a lower 28-day mortality rate with 32 fewer individuals per 1,000 who succumbed when treated with tocilizumab plus standard care, compared with standard care alone or placebo (11), and the third revealed a 22% 28-day mortality rate (12).

On the other hand, there is evidence to indicate that tocilizumab is ineffective in some cases of COVID-19, as it has been hypothesized that early drug administration is probably more beneficial (13). The World Health Organization (WHO) recommends the use of tocilizumab if inflammation is evident and a patient has severe or critical COVID-19 (14). More specifically tocilizumab is recommended in patients who have rapidly increasing oxygen needs and systemic inflammation (14). Tocilizumab is a potent anti-inflammatory drug that has been shown to reduce C-reactive protein (CRP) levels, although not always with a therapeutic effect. Other clinical parameters, such as the degree of hypoxia, may be a crucial factor in the decision of whether to escalate treatment or in determining prognosis (15). The present study aimed to describe in detail the characteristics of patients who received this agent and to identify determinants of mortality and other unfavorable outcomes.

Patients and methods

Study design. The present study was a single-center retrospective study on patients with COVID-19 admitted to the Department of Infectious Diseases-COVID-19 Unit of Laiko General Hospital (Athens, Greece) between September 21, 2020 and April 15, 2022. The study was conducted in line with the Declaration of Helsinki and obtained approval by the Institutional Review Board of Laiko General Hospital (protocol no. 765/12-2021). Written informed was obtained from all patients. The following criteria were required for inclusion in the study: A polymerase chain reaction diagnosis of COVID-19, a WHO clinical progression scale score ≥5, and tocilizumab treatment in accordance with the WHO recommendations (13). Some of the participants had a follow-up appointment 3 months after their admission to the post-COVID-19 outpatient clinic of Laiko General Hospital, and if that was not possible, a telephone call was made to determine the 90-day mortality rate. The exclusion criteria were an age <18 years and a lack of available data on survival at 3 months post-diagnosis.

Investigations. Demographics, vaccination status against SARS-CoV-2 and the Charlson comorbidity index (CCI)

Table I. Demographics of the study population.

Parameter	Mean/no. of patients	SD/%
Age (mean ± SD)	62.43	13.47
Sex, number and percentage	174	
Male	121	69.5
Female	53	30.5
Type of treatment		
Remdesivir	174	
Yes	172	98.9
No	2	1.1
Dexamethasone	174	
Yes	173	99.4
No	1	0.6
Anticoagulants	174	
No	5	2.9
Yes	169	97.1
Anticoagulants	169	
Prophylactic dose	161	95.2
Therapeutic dose	8	4.8
Outcome		
Intubation	174	
Yes	58	33.3
No	116	66.7
Mortality at 90 days	174	
Yes	61	35.1
No	113	64.9
Vaccination status	174	
Fully vaccinated	20	11.5
Unvaccinated	154	88.5

SD, standard deviation.

Table II. Univariate analysis (outcome, mortality).

Parameter	Survivors	Non-survivors	P-value
Age (years)	57.88±12.86	70.84±10.17	**0.01**
Sex			**0.01**
Male	86	35	
Female	27	26	
Type of treatment			
Remdesivir			0.999
Yes	112	60	
No	1	1	
Dexamethasone			0.999
Yes	112	61	
No	1	0	
Anticoagulants			0.65
Yes	109	60	
No	4	1	
Vaccination status			0.07
Fully vaccinated	9	11	
Unvaccinated	104	50	
CCI	1.96±1.71	3.49±1.46	**0.01**
Hb (g/dl)	13.77±1.38	13.66±1.89	0.67
WBC (k/μl)	8.44±8.03	9.50±14.58	0.67
Neutrophils (k/μl)	6.54±3.61	6.42±2.92	0.73
Lymphocytes (k/μl)	1.62±6.45	2.31±10.80	0.57
IGs (k/μl)	0.11±0.26	0.09± 0.16	0.97
PLTs (k/μl)	227.71±83.86	195.56±66.72	**0.01**
D-dimers (μg/ml)	2.18±4.40	2.67±4.86	0.19
Creatinine (mg/dl)	1.20±1.69	1.33±1.73	0.22
AST (U/l)	52.43±36.82	48.36±26.65	0.72
ALT (U/l)	50.69±49.64	35.67± 27.09	**0.02**
ALP (U/l)	72.96± 35.97	72.42±28.58	0.40
GGT (U/l)	77.34 ±80.91	60.78±72.16	**0.03**
LDH (U/l)	447.38±175.60	442±185.39	0.82
CRP (mg/l)	127.83 ±82.96	108.74 ±79.66	0.09
Fibrinogen (mg/dl)	634.16±162.36	602.92±155.85	0.22
Ferritin (ng/ml)	1,258.25±1,629.29	1,527.11±1,821.12	0.42
Albumin (g/l)	37.77±4.63	36.48±5.61	0.15
NLR	9.19±11.89	9.25±8.53	0.75
PLR	329.07±526.24	273.04±201.45	0.52
CAR	3.47±2.51	3.05±2.55	0.15
Chest X-ray score	9.15±2.99	9.64±3.17	0.13

Values in bold font indicate statistically significant differences (P<0.05). ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCI, Charlson comorbidity index; CRP, C-reactive protein; CAR, CRP-to-albumin ratio; GGT, gamma glutamyl-transferase; Hb, hemoglobin; IGs, immature granulocytes; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; PLTs, platelets; PLR, platelet-to-lymphocyte ratio; WBC, white blood cell.

were recorded. Hemoglobin levels, white blood cell (WBC) count, blood neutrophil, lymphocyte and immature granulocyte counts, neutrophil-to-lymphocyte ratio, the number of platelets (PLTs), platelet-to-lymphocyte ratio, CRP and serum albumin levels, CRP-to-albumin ratio (CAR), serum lactate dehydrogenase (LDH), d-dimer, ferritin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase and gamma glutamyl-transferase (GGT) levels were recorded upon admission. In addition, the Modified Chest X-ray Scoring System was calculated for chest X-ray

Table III. Univariate analysis (outcome, intubation).

Parameter	Non-intubated	Intubated	P-value
Age (years)	59.80±14.36	67.67±9.62	**0.01**
Sex			**0.03**
Male	86	34	
Female	30	24	
Type of treatment			
Remdesivir			0.55
Yes	114	58	
No	2	0	
Dexamethasone			0.33
Yes	116	57	
No	0	1	
Anticoagulants			0.66
Yes	112	57	
No	4	1	
Vaccination status			0.23
Fully vaccinated	11	9	
Unvaccinated	105	49	
CCI	2.15±1.82	3.19±1.49	**0.01**
Hb (g/dl)	13.71±1.45	13.78±1.82	0.92
WBC (k/μl)	8.34±7.95	9.74±14.91	0.28
Neutrophils (k/μl)	6.42±3.58	6.66±2.95	0.24
Lymphocytes (k/μl)	1.63±6.36	2.33±11.08	0.10
IGs (k/μl)	0.12±0.27	0.09±0.11	0.38
PLTs (k/μl)	225.16±84.33	198.91±66.39	0.06
D-dimers (μg/ml)	2.24±4.36	2.58±4.98	0.82
Creatinine (mg/dl)	1.22±1.67	1.30±1.76	0.49
AST (U/l)	52.25±36.86	48.52±25.92	0.91
ALT (U/l)	50.28±49.98	35.71±24.22	0.08
ALP (U/l)	73.44±35.96	71.45±28.25	0.62
GGT (U/l)	69.47±62.97	75.81±102.39	0.55
LDH (U/l)	439.87±174.25	457±188.01	0.51
CRP (mg/l)	122.67±83.17	118.07±80.52	0.70
Fibrinogen (mg/dl)	619.58±166.74	631.20±147.58	0.65
Ferritin (ng/ml)	1,307.16±1,716.38	1,444.05±1,675.10	0.44
Albumin (g/l)	37.69±4.54	36.57±5.84	0.22
NLR	8.77±11.69	10.09±8.80	0.09
PLR	320.92±519.88	286.16±206.57	0.93
CAR	3.34±2.52	3.28±2.55	0.63
Chest X-ray score	9.28±3.30	9.41±2.94	0.43

Values in bold font indicate statistically significant differences (P<0.05). ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCI, Charlson comorbidity index; CRP, C-reactive protein; CAR, CRP-to-albumin ratio; GGT, gamma glutamyl-transferase; Hb, hemoglobin; IGs, immature granulocytes; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; PLTs, platelets; PLR, platelet-to-lymphocyte ratio; WBC, white blood cell.

upon admission by two experienced radiologists, as previously described (16). Charts were evaluated for the implementation of intubation and all-cause mortality rates at 90 days.

Statistical analysis. Continuous variables are presented as the mean (standard deviation). The assessment of the normal distribution of variables was performed with the use the Kolmogorov-Smirnov test. The comparison of normally distributed variables was performed using an independent samples Student's t-test on variables with two groups and not normally distributed variables were examined using an unpaired non-parametric two-tailed Mann-Whitney test.

Table IV. Cox regression multivariable analysis (outcome, mortality).

			95% CI for Exp(B)	
Parameter	P-value	Exp(B)	Lower	Upper
Age	0.01	1.045	1.010	1.081
Sex	0.17	1.436	0.851	2.425
PLTs (k/μl)	0.07	0.996	0.992	1.000
ALT (U/l)	0.82	0.999	0.990	1.008
GGT (U/l)	0.88	1.000	0.996	1.003
CCI	0.75	1.038	0.820	1.316

ALT, alanine aminotransferase; CdCI, Charlson comorbidity index; GGT, gamma glutamyl-transferase; PLTs, platelets; 95% CI, 95% confidence interval.

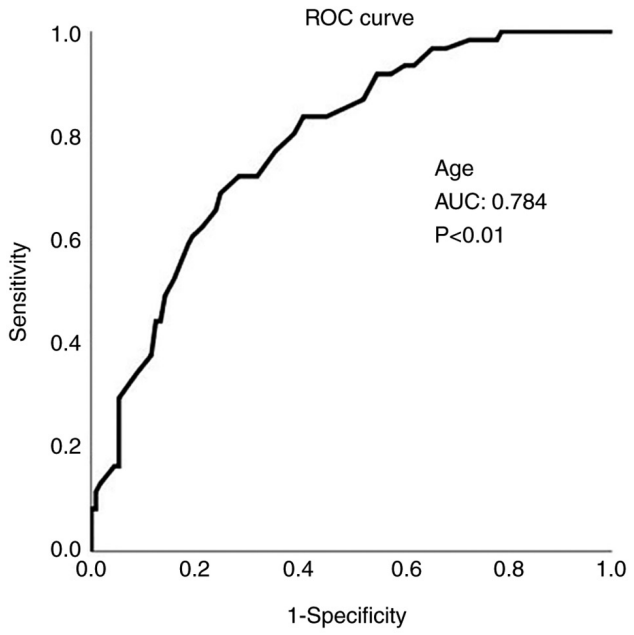

Figure 1. ROC curve for age predicting mortality in tocilizumab-treated patients. AUC, 0.784 (P<0.01). AUC, area under the curve; ROC, receiver operative characteristic.

Categorical variables were examined using the Fisher's exact test or the Chi-squared test and are presented as absolute numbers (frequency, percentage). The CCI data were numerically recorded. To find predictors of event(s) (event=intubation, or mortality at 90 days), statistically significant factors were subsequently examined using Cox proportional hazards multivariate regression analysis. The quality of fit of the log-likelihood ratio was evaluated. The Kaplan-Meier method with log-rank (Mantel-Cox) test was used to plot and analyze survival curves utilizing significant variables and specific cut-offs. The discriminative ability of significant variables was evaluated by using the area under the receiver operating characteristic curve (ROC). Participants were censored at 90 days. Values of P<0.05 were considered to indicate statistically significant differences. Statistical analysis was conducted using IBM SPSS-Statistics version 26.0 (IBM Corp.).

Table V. Cox regression multivariable analysis (outcome, intubation).

			95% CI for Exp(B)	
Parameter	P-value	Exp(B)	Lower	Upper
Age	0.286	1.018	0.985	1.052
CCI	0.372	1.108	0.885	1.388
Sex	0.231	1.382	0.814	2.348

CCI, Charlson comorbidity index; 95% CI, 95% confidence interval.

Results

In total, 174 subjects (121 males; mean age, 62.43±13.47 years) fulfilling the inclusion criteria were included. Among the 174 participants, 58 (33.3%) were intubated. From the 174 individuals analyzed, 113 were alive after 90 days (survivors), and 61 had succumbed (non-survivors). The mortality rate was 35.1% (61/174). The demographics and baseline data of the study population are presented in Table I.

The non-survivors were older, mostly females and had a higher CCI score. At the evaluation upon admission to the hospital unit, the survivors presented with higher levels of ALT and GGT and with a greater number of PLTs (P<0.05; Table II). The patients that were intubated were also older, mostly females, and had a higher CCI score (P<0.05; Table III).

All parameters with significant differences in the univariate analysis were analyzed using the Cox proportional hazards multivariate regression analysis. The outcome was all-cause mortality, and cases were censored at 90 days. The only independent predictor of mortality found was age (P<0.05; Table IV).

In addition, age was also found to be a significant predictor of mortality using ROC analysis (Fig. 1). An age >64.5 years predicted mortality with 72.1% sensitivity and 71.7% specificity (AUC, 0.784). Kaplan-Meier survival analysis based on cut-off values for age (>64.5 years and ≤64.5 years) revealed a worse survival in subjects with an age >64.5 years (log-rank test for trend, P<0.05; Fig. 2). Furthermore, Cox proportional hazards multivariate regression analysis with intubation as the outcome did not identify any independent factors predicting intubation (Table V).

Of note, a sub-analysis regarding sex was performed, which revealed that the male survivors were younger, had lower chest X-ray scores, greater PLTs values and serum albumin, and lower values of CCI and creatinine (P<0.05; Table VI).

Cox proportional hazards multivariate regression analysis with mortality as the outcome identified PLTs as an independent factor predicting mortality in males (Table VII). In addition, the male patients that were intubated were older, had higher values of CCI, creatinine and ferritin and lower values of platelets (P<0.05; Table VI). Moreover, Cox proportional hazards multivariate regression analysis with intubation as the

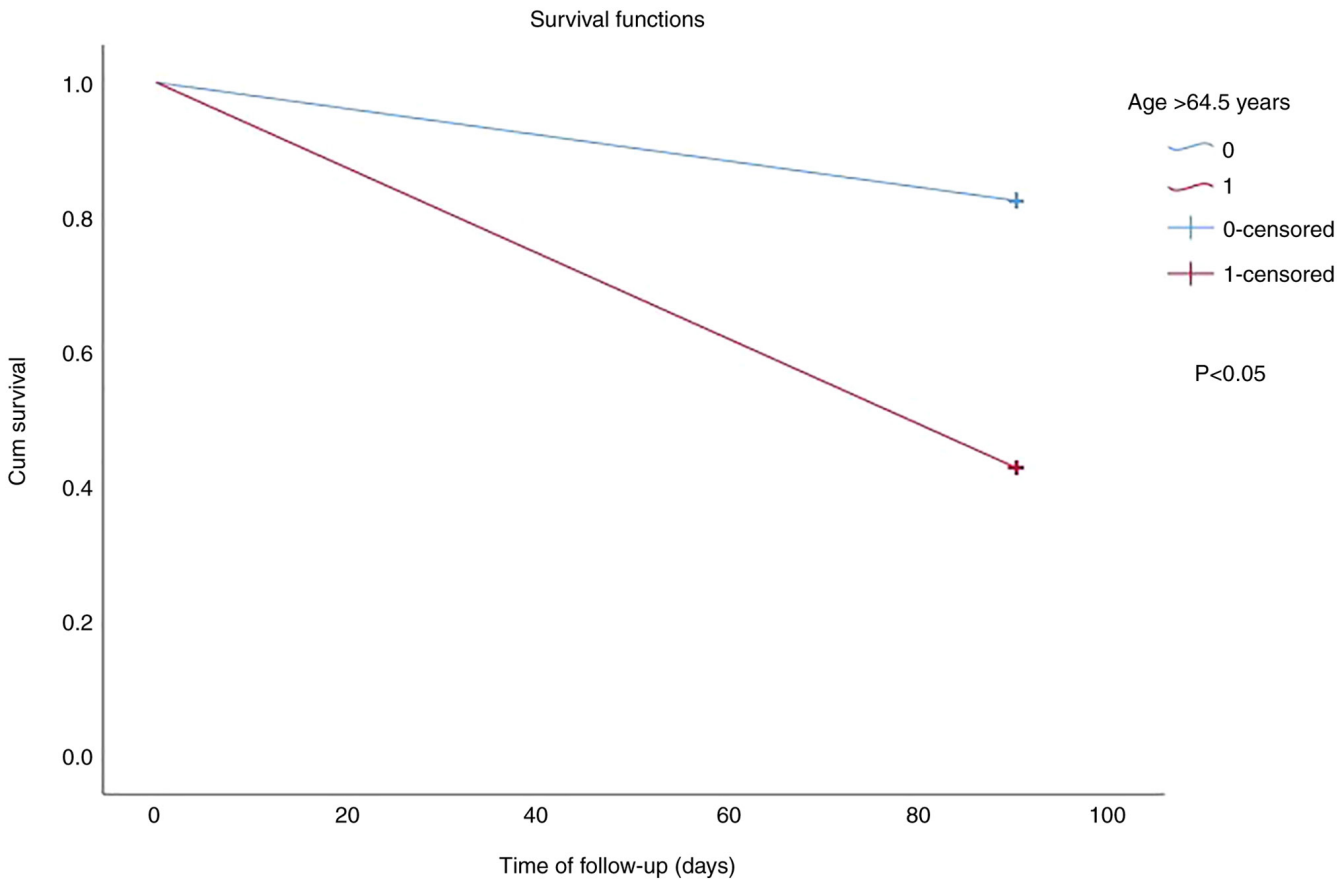

Figure 2. Kaplan-Meier survival curve. A significantly worse survival was observed for patients with an age >64.5 years (P<0.05).

outcome, identified PLTs as an independent factor predicting intubation in male patients (Table VII).

As regards the female patients, the survivors were younger, had lower values of CCI, and greater values of GGT and CAR compared to the non-survivors (P<0.05). In addition, female patients that were intubated had lower values of lymphocytes compared to those that were not intubated (P<0.05; Table VIII). Furthermore, Cox proportional hazards multivariate regression analysis with mortality as the outcome did not identify any independent factor predicting mortality in female patients (Table IX).

Discussion

The mortality rate found in the present study was relatively higher compared to the rates demonstrated in randomized controlled trials of tocilizumab and in meta-analyses mentioning a pooled mortality prevalence of 20-30% (17-21). One of the main findings of the present study was that among all patients treated with tocilizumab for COVID-19-associated pneumonia, age was the only independent factor predicting 90-day mortality.

Previous research has indicated that age is a major risk factor for mortality in SARS-CoV-2 infection (22-24). As a result of age-related hematopoietic mosaic chromosomal changes that decrease immunity, age has been revealed to be the main risk factor for infections and the accompanying mortality (25). Moreover, differences in lung structure, muscular atrophy, poor airway clearance, diminished lung reserve, diminished resistance to infections, the increased expression of angiotensin converting enzyme (ACE)-2, particularly among the elderly receiving ACE inhibitors and angiotensin II receptor blockers, along with prior exposure to circulating coronaviruses with reduced neutralizing capacity to SARS-CoV-2, may all contribute to an increased vulnerability of elderly individuals to this infection and poor outcomes (26). Similar to the findings of the present study, age has been reported as a predictor of mortality in patients receiving tocilizumab for SARS-CoV-2 infection in other studies (27-33).

Another notable finding of the present study was that the mortality rate was higher among female patients compared to male patients. However, sex was not identified as an independent factor predicting mortality according to the multivariate analysis, as has been shown in previous research (34). It has been reported that males have significantly higher rates of adverse events and mortality due to COVID-19 (35). Biological sex differences manifest as differences in the balance between inflammation and tissue healing following the resolution of infection, differences in the time of pathogenesis, differences in innate viral control and adaptive immune responses, and a difference in vulnerability to infection (36). These disparities in sex are most likely pathogen-specific and complex in nature. Thus far, changes in immune function linked with the X chromosome, the impact of sex hormones and sex-related behavioral and

Table VI. Univariate analysis for male patients.

Outcome, mortality

Parameter	Survivors	Non-survivors	P-value
Age (years)	57.06±12.61	71.09±10.47	**0.001**
CCI	1.92±1.75	3.49±1.54	**0.001**
Hb (g/dl)	14.09±1.32	13.88±2.11	0.58
WBC (k/μl)	7.82±3.64	10.93±19.06	0.60
Neutrophils (k/μl)	6.60±3.63	6.62±2.95	0.98
Lymphocytes (k/μl)	1.00±0.97	3.27±14.26	0.50
IGs (k/μl)	0.11±0.26	0.11±0.18	0.34
PLTs (k/μl)	231.98±84.80	182.94±59.26	**0.002**
D-dimers (μg/ml)	2.22±4.57	3.68±6.23	0.056
Creatinine (mg/dl)	1.35±1.91	1.45±1.33	**0.005**
AST (U/l)	52.60±37.66	51.66±30.19	0.95
ALT (U/l)	53.19±53.94	38.91±30.24	0.12
ALP (U/l)	69.91±33.88	71.74±31.85	0.52
GGT (U/l)	78.05±83.05	62.09±55.64	0.30
LDH (U/l)	443.36±180.05	452.50±202.18	0.82
CRP (mg/l)	130.06±80.86	118.26±71.19	0.59
Fibrinogen (mg/dl)	650.70±152.52	626.38±146.08	0.42
Ferritin (ng/ml)	1,377.47±1,760.41	1,929.71±1,999.20	0.90
Albumin (g/l)	37.96±4.71	35.78±5.56	**0.047**
NLR	9.98±13.25	10.11±7.83	0.97
PLR	359.62±592.21	273.01±190.27	0.40
CAR	3.52±2.37	3.68±2.61	1.00
Chest X-ray score	8.80±3.01	9.71±3.11	**0.04**

Outcome, intubation

Parameter	Non-intubated	Intubated	P-value
Age (years)	58.60±13.71	67.56±11.00	**0.001**
CCI	2.05±1.81	3.21±3.62	**0.001**
Hb (g/dl)	14.02±1.40	14.06±2.00	0.88
WBC (k/μl)	7.69±3.60	11.36±19.30	0.19
Neutrophils (k/μl)	6.45±3.57	7.01±3.05	0.42
Lymphocytes (k/μl)	1.00±0.76	3.32±14.47	0.26
IGs (k/μl)	0.11±0.28	0.09±0.10	0.07
PLTs (k/μl)	230.47±84.85	185.32±60.69	**0.002**
D-dimers (μg/ml)	2.26±4.54	3.61±6.36	0.29
Creatinine (mg/dl)	1.38±1.90	1.37±1.34	**0.023**
AST (U/l)	53.14±37.80	50.26±29.40	0.85
ALT (U/l)	53.80±54.41	36.91±25.75	0.09
ALP (U/l)	71.02±33.71	68.94±32.24	0.65
GGT (U/l	68.94±58.84	82.85±109.53	0.89
LDH (U/l)	439.13±178.75	463.94±204.95	0.57
CRP (mg/l)	125.50±81.12	129.57±70.76	0.79
Fibrinogen (mg/dl)	641.11±157.8	650.91±133.66	0.75
Ferritin (ng/ml)	1,374.27±1,765.06	1,954.02±1,990.64	**0.036**
Albumin (g/l)	37.72±4.57	36,.20±6.05	0.17
NLR	9.69±13.15	10.88±7.98	0.62
PLR	358.09±589.22	274.33±190.30	0.41
CAR	3.41±2.38	3.95±2.58	0.40

Table VI. Continued.

Outcome, intubation			
Parameter	Non-intubated	Intubated	P-value
Chest X-ray score	8.81±3.04	9.71±3.04	0.066

Values in bold font indicate statistically significant differences (P<0.05). ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCI, Charlson comorbidity index; CRP, C-reactive protein; CAR, CRP-to-albumin ratio; GGT, gamma glutamyl-transferase; Hb, hemoglobin; IGs, immature granulocytes; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; PLTs, platelets; PLR, platelet-to-lymphocyte ratio; WBC, white blood cell.

Table VII. Cox regression multivariable analysis for male patients.

Outcome, mortality				
			95% CI for Exp(B)	
Parameter	P-value	Exp(B)	Lower	Upper
Age (years)	0.052	1.056	0.999	1.115
X-ray score	0.393	1.055	0.933	1.193
CCI	0.959	1.010	0.686	1.488
PLTs (K/μl)	**0.048**	0.994	0.987	0.999
Creatinine (mg/dl)	0.868	0.981	0.778	1.236
Albumin (g/l)	0.828	1.009	0.929	1.096

Outcome, intubation				
			95% CI for Exp(B)	
Parameter	P-value	Exp(B)	Lower	Upper
Age (years	0.244	1.027	0.982	1.074
CCI	0.781	1.049	0.750	1.467
PLTs (K/μl)	**0.025**	0.994	0.988	0.999
Creatinine (mg/dl)	0.686	0.947	0.727	1.233
Ferritin (ng/ml)	0.367	1.000	0.997	0.999

Values in bold font indicate statistically significant differences (P<0.05). 95% CI, 95% confidence interval; CCI, Charlson comorbidity index; PLTs, platelets.

sociocultural variables have been hypothesized to explain male-female discrepancies in SARS-CoV-2 infection. For example, the existence of monoallelic vs. biallelic ACE2 and Toll-like receptor 7 genes on the X chromosome may help to explain the greater risk of COVID-19 infection in males compared to females (37).

Several studies have reported laboratory parameters, such as eosinophils, lymphocytes, PLTs, immature granulocytes, ferritin and liver enzymes as biomarkers of poor outcomes in patients with COVID-19 (36,38-42). Of note, only a few studies have evaluated laboratory data as biomarkers of poor outcomes in tocilizumab-treated patients with COVID-19 (29-34,43-45). Some studies have examined the role of laboratory parameters on specific days following the tocilizumab administration as potential markers of mortality (31,45). According to the aforementioned studies, d-dimer levels (29), the WBC count (30), LDH levels (32,45), procalcitonin levels (33), ferritin (43), AST levels (44), CRP levels (34), lymphocyte count (44,45) and the number of PLTs (31,34,44), have all been identified as predictors of poor outcomes in tocilizumab-treated patients with COVID-19. Of note, the value of PTLs upon admission and following the tocilizumab administration was found to be

Table VIII. Univariate analysis for female patients.

Outcome, mortality

Parameter	Survivors	Non-survivors	P-value
Age (years)	60.52±13.55	71.00±10.10	**0.02**
CCI	2.07±1.61	3.59±1.44	**0.001**
Hb (g/dl)	12.76±1.07	13.26±1.64	0.18
WBC (k/μl)	10.39±15.13	7.49±3.00	0.98
Neutrophils (k/μl)	6.36±3.64	6.06±2.90	0.97
Lymphocytes (k/μl)	3.60±13.11	1.02±0.48	0.41
IGs (k/μl)	0.14±0.23	0.07±0.13	0.18
PLTs (k/μl)	213.77±80.72	211.78±72.07	0.90
D-dimers (μg/ml)	2.05±3.86	1.36±1.43	0.60
Creatinine (mg/dl)	0.74±0.19	1.24±2.13	0.26
AST (U/l)	51.89±34.66	43.33±20.53	0.56
ALT (U/l)	42.74±31.88	30.59±21.87	0.15
ALP (U/l)	82.70±41.11	73.58±23.41	0.97
GGT (U/l)	75.07±75.15	56.96±90.43	**0.04**
LDH (U/l)	460.19±163.15	418.96±167.67	0.14
CRP (mg/l)	120.73±90.55	92.91±89.32	10.13
Fibrinogen (mg/dl)	579.47±184.11	562,42±168.31	0.72
Ferritin (ng/ml)	882.96±1,062.10	953.92±1,393.28	0.52
Albumin (g/l)	37.22±4.45	37.53±5.56	0.84
NLR	6.67±5.11	7.93±9.29	0.53
PLR	229.21±162.91	270.22±215.71	0.36
CAR	3.23±2.75	1.92±2.14	**0.03**
Chest X-ray score	10.31±2.69	9.56±3.25	0.29

Outcome, intubation

Parameter	Non-intubated	Intubated	P-value
Age (years)	64.10±16.03	67.83±7.45	0.26
CCI	2.57±1.94	3.17±1.30	0.16
Hb (g/dl)	12.71±1.26	13.39±1.48	0.07
WBC (k/μl)	10.14±14.42	7.44±2.76	0.78
Neutrophils (k/μl)	6.24±3.64	6.17±2.8	0.67
Lymphocytes (k/μl)	3.42±12.43	0.93±0.42	**0.043**
IGs (k/μl)	0.13±0.22	0.08±0.13	0.51
PLTs (k/μl)	208.28±80.66	218.17±70.56	0.32
D-dimers (μg/ml)	2.11±3.73	1.21±1.17	0.37
Creatinine (mg/dl)	0.78±0.23	1.25±2.26	0.81
AST (U/l	48.87±34.01	46.04±20.37	0.65
ALT (U/l	38.80±31.68	34.00±22.31	0.92
ALP (U/l)	80.90±41.24	75.00±21.55	0.52
GGT (U/l)	66.48±75.10	65.83±92.7	0.79
LDH (U/l)	433.27±167.32	447.46±165.69	0.68
CRP (mg/l	110.84±90.31	101.78±91.72	0.56
Fibrinogen (mg/dl)	545.59±181.49	602.91±164.46	0.24
Ferritin (ng/ml)	1,075.93±1,577.99	721.58±582.44	0.70
Albumin (g/l)	37.43±4.55	37.25±5.57	0.91
NLR	5.96±4.42	8.97±9.90	0.10
PLR	206.39±139.84	302.93±230.84	0.06
CAR	2.93±2.76	2.20±2.21	0.21

Table VIII. Continued.

Outcome, intubation			
Parameter	Non-intubated	Intubated	P-value
Chest X-ray score	10.69±2.02	9.00±3.68	0.08

Values in bold font indicate statistically significant differences (P<0.05). ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCI, Charlson comorbidity index; CRP, C-reactive protein; CAR, CRP-to-albumin ratio; GGT, gamma glutamyl-transferase; Hb, hemoglobin; IGs, immature granulocytes; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; PLTs, platelets; PLR, platelet-to-lymphocyte ratio; WBC, white blood cell.

Table IX. Cox regression multivariable analysis for female patients.

Outcome, mortality				
			95% CI for Exp(B)	
Parameter	P-value	Exp(B)	Lower	Upper
CCI	0.891	0.960	0.533	1.728
Age (years)	0.317	1.040	0.963	1.123
CAR	0.413	0.904	0.710	1.151
GGT (U/l)	0.843	0.999	0.994	1.005

CCI, Charlson comorbidity index; CAR, C-reactive protein-to-albumin ratio; GGT, gamma glutamyl-transferase; 95% CI, 95% confidence interval.

significantly associated with mortality in the study by Sarabia De Ardanaz *et al* (31).

The present study did not identify any independent laboratory factors predicting intubation or mortality in the population examined. However, when analyzing females and males who had a difference in mortality rate separately, it was identified that the value of PLTs was the only independent factor predicting intubation and 90-day mortality in male patients, and the lymphocyte count was the only factor associated with intubation in female patients. Several clinical studies have found that increased platelet activation leads to platelet deposition in injured pulmonary blood arteries, and thrombocytopenia is a common characteristic of SARS-CoV-2 infection (46,47). Thrombocytopenia is a major predictor of a poor prognosis. PLTs in patients with SARS-CoV-2 are inversely linked with soluble vascular cell adhesion molecule-1 (sVCAM-1) levels. sVCAM-1 is involved in adhesion and chemotaxis, and it leads to early vascular damage and T-cell inhibition. The poor outcome observed may be explained by vascular damage or immunosuppression (48).

The present study is one of a handful of studies evaluating laboratory data as biomarkers of poor outcomes in tocilizumab-treated patients with COVID-19 and, to the best of our knowledge, the first to mention PLTs as an independent factor predicting intubation and 90-day mortality in male patients treated with tocilizumab and the lymphocyte count as the only factor associated with intubation in female patients treated with tocilizumab.

The present study has certain limitations which should be mentioned. It was of a retrospective design, and there was no control group. Furthermore, it is possible that the negative results obtained in the present study (all-cause mortality) may be attributable to other etiologies in addition to severe COVID-19 (thromboembolism, sepsis, or coexisting diseases). The advantages of the present study were the relatively large number of tocilizumab-treated patients, the reliable follow-up data and the availability of 90-day data. Another strong point of the study was that participants were patients with COVID-19 admitted between September 21, 2020 and April 15, 2022, covering the periods of alpha, delta and omicron variant predominance.

In conclusion, in the present retrospective study, mortality occurred in 35.1% of the tocilizumab-treated COVID-19 patients, with a greater rate of mortality observed among females. The only independent prognosticator of mortality in the study population was age. In addition, the value of PLTs was an independent factor predicting intubation and 90-day mortality in male patients treated with tocilizumab, and the lymphocyte count was the only factor associated with intubation in female patients treated with tocilizumab. These data may be used to identify patient subpopulations responding to therapy in prospective clinical trials investigating the efficacy of treatment with tocilizumab.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DB, GK and VEG conceptualized the study. VEG, DB, PMV, GK, IrE, SS, SM, OK, IoE, MT, AB, CVP and AA advised on patient care and medical treatment, obtained patient data, wrote and prepared the draft of the manuscript and made substantial contributions to the acquisition and interpretation of data. DAS, PP, AG and NVS analyzed the data and provided critical revisions. VEG and NVS confirm the authenticity of all the data. All authors contributed to manuscript revision. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted in line with the Declaration of Helsinki and obtained approval by the Institutional Review Board of Laiko General Hospital (protocol no. 765/12-2021). Written informed was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

DAS is the Editor-in-Chief for the journal, but had no personal involvement in the reviewing process, or any influence in terms of adjudicating on the final decision, for this article. The other authors declare that they have no competing interests.

References

1. World Health Organization: WHO coronavirus (COVID-19) dashboard. https://covid19.who.int/. Accessed August 28, 2022.
2. Naleway AL, Groom HC, Crawford PM, Salas SB, Henninger ML, Donald JL, Smith N, Thompson MG, Blanton LH, Bozio CH and Azziz-Baumgartner E: Incidence of SARS-CoV-2 infection, emergency department visits, and hospitalizations because of COVID-19 among persons aged ≥12 years, by COVID-19 vaccination status-oregon and washington, July 4-September 25, 2021. MMWR Morb Mortal Wkly Rep 70: 1608-1612, 2021.
3. Paidas MJ, Sampath N, Schindler EA, Cosio DS, Ndubizu CO, Shamaladevi N, Kwal J, Rodriguez S, Ahmad A, Kenyon NS and Jayakumar AR: Mechanism of Multi-organ injury in experimental COVID-19 and its inhibition by a small molecule peptide. Front Pharmacol 13: 864798, 2022.
4. Darif D, Hammi I, Kihel A, El Idrissi Saik I, Guessous F and Akarid K: The pro-inflammatory cytokines in COVID-19 pathogenesis: What goes wrong? Microb Pathog 153: 104799, 2021.
5. Mogensen TH: Pathogen recognition and inflammatory signaling in innate immune defenses. Clin Microbiol Rev 22: 240-273, 2009.
6. Chen LYC, Hoiland RL, Stukas S, Wellington CL and Sekhon MS: Confronting the controversy: Interleukin-6 and the COVID-19 cytokine storm syndrome. Eur Respir J 56: 2003006, 2020.
7. Fraenkel L, Bathon JM, England BR, St Clair EW, Arayssi T, Carandang K, Deane KD, Genovese M, Huston KK, Kerr G, *et al*: 2021 American college of rheumatology guideline for the treatment of rheumatoid arthritis. Arthritis Care Res (Hoboken) 73: 924-939, 2021.
8. RECOVERY Collaborative Group: Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): A randomised, controlled, open-label, platform trial. Lancet 397: 1637-1645, 2021.
9. REMAP-CAP Investigators, Gordon AC, Mouncey PR, Al-Beidh F, Rowan KM, Nichol AD, Arabi YM, Annane D, Beane A, van Bentum-Puijk W, *et al*: Interleukin-6 receptor antagonists in critically ill patients with Covid-19. N Engl J Med 384: 1491-1502, 2021.
10. Berardicurti O, Ruscitti P, Ursini F, D'Andrea S, Ciaffi J, Meliconi R, Iagnocco A, Cipriani P and Giacomelli R: Mortality in tocilizumab-treated patients with COVID-19: A systematic review and meta-analysis. Clin Exp Rheumatol 38: 1247-1254, 2020.
11. Ghosn L, Chaimani A, Evrenoglou T, Davidson M, Graña C, Schmucker C, Bollig C, Henschke N, Sguassero Y, Nejstgaard CH, *et al*: Interleukin-6 blocking agents for treating COVID-19: A living systematic review. Cochrane Database Syst Rev 3: CD013881, 2021.
12. WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group, Shankar-Hari M, Vale CL, Godolphin PJ, Fisher D, Higgins JPT, Spiga F, Savovic J, Tierney J, Baron G, *et al*: Association between administration of IL-6 antagonists and mortality among patients hospitalized for COVID-19: A meta-analysis. JAMA 326: 499-518, 2021.
13. San-Juan R, Fernández-Ruiz M, López-Medrano F, Carretero O, Lalueza A, Maestro de la Calle G, Pérez-Jacoiste Asín MA, Bueno H, Caro-Teller JM, Catalán M, *et al*: Analysis of the factors predicting clinical response to tocilizumab therapy in patients with severe COVID-19. Int J Infect Dis 117: 56-64, 2022.
14. World Health Organization: Therapeutics and COVID-19. Living guideline, July 6, 2021. https://apps.who.int/iris/bitstream/handle/10665/342368/WHO-2019-nCoV-therapeutics-2021.2-eng.pdf. Accessed August 28, 2022.
15. Salvarani C, Dolci G, Massari M, Merlo DF, Cavuto S, Savoldi L, Bruzzi P, Boni F, Braglia L, Turrà C, *et al*: Effect of tocilizumab vs standard care on clinical worsening in patients hospitalized with COVID-19 pneumonia: A randomized clinical trial. JAMA Intern Med 181: 24-31, 2021.
16. Setiawati R, Widyoningroem A, Handarini T, Hayati F, Basja AT, Putri ARDS, Jaya MG, Andriani J, Tanadi MR and Kamal IH: Modified chest X-Ray scoring system in evaluating severity of COVID-19 patient in Dr. Soetomo general hospital Surabaya, Indonesia. Int J Gen Med 14: 2407-2412, 2021.
17. Zhang J, Chen C, Yang Y and Yang J: Effectiveness of tocilizumab in the treatment of hospitalized adults COVID-19: A systematic review and meta-analysis. Medicine (Baltimore) 101: e28967, 2022.
18. Peng J, She X, Mei H, Zheng H, Fu M, Liang G, Wang Q and Liu W: Association between tocilizumab treatment and clinical outcomes of COVID-19 patients: A systematic review and meta-analysis. Aging (Albany NY) 14: 557-571, 2022.
19. Luo L, Luo T, Du M, Mei H and Hu Y: Efficacy and safety of tocilizumab in hospitalized COVID-19 patients: A systematic review and meta-analysis. J Infect 84: 418-467, 2022.
20. Vela D, Vela-Gaxha Z, Rexhepi M, Olloni R, Hyseni V and Nallbani R: Efficacy and safety of tocilizumab versus standard care/placebo in patients with COVID-19; a systematic review and meta-analysis of randomized clinical trials. Br J Clin Pharmacol 88: 1955-1963, 2022.
21. Piscoya A, Parra Del Riego A, Cerna-Viacava R, Rocco J, Roman YM, Escobedo AA, Pasupuleti V, White CM and Hernandez AV: Efficacy and harms of tocilizumab for the treatment of COVID-19 patients: A systematic review and meta-analysis. PLoS One 17: e0269368, 2022.
22. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, *et al*: Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. Lancet 395: 1054-1062, 2020.

23. Kokkoris S, Gkoufa A, Maneta E, Doumas G, Mizi E, Georgakopoulou VE, Sigala I, Dima E, Papachatzakis I, Ntaidou TK, *et al*: Older adults with severe coronavirus disease 2019 admitted to intensive care unit: Prevalence, characteristics and risk factors for mortality. Minerva Anestesiol: Apr 13, 2022 (Epub ahead of print).
24. Gkoufa A, Maneta E, Ntoumas GN, Georgakopoulou VE, Mantelou A, Kokkoris S and Routsi C: Elderly adults with COVID-19 admitted to intensive care unit: A narrative review. World J Crit Care Med 10: 278-289, 2021.
25. Zekavat SM, Lin SH, Bick AG, Liu A, Paruchuri K, Wang C, Uddin MM, Ye Y, Yu Z, Liu X, *et al*: Hematopoietic mosaic chromosomal alterations increase the risk for diverse types of infection. Nat Med 27: 1012-1024, 2021.
26. Georgakopoulou VE, Papalexis P, Sanos C, Bitsani A, Garmpi A, Damaskos C, Garmpis N, Gkoufa A, Chlapoutakis S, Sklapani P, *et al*: Asymptomatic SARS-CoV-2 infection in an unvaccinated 97-year-old woman: A case report. Biomed Rep 15: 107, 2021.
27. AlQahtani H, AlBilal S, Mahmoud E, Aldibasi O, Alharbi A, Shamas N, Alsaedy A, Owaidah K, Alqahtani FY, Aleanizy FS, *et al*: Outcomes associated with tocilizumab with or without corticosteroid versus dexamethasone for treatment of patients with severe to critical COVID-19 pneumonia. J Infect Public Health 15: 36-41, 2022.
28. Duarte-Millán MA, Mesa-Plaza N, Guerrero-Santillán M, Morales-Ortega A, Bernal-Bello D, Farfán-Sedano AI, García de Viedma-García V, Velázquez-Ríos L, Frutos-Pérez B, De Ancos-Aracil CL, *et al*: Prognostic factors and combined use of tocilizumab and corticosteroids in a Spanish cohort of elderly COVID-19 patients. J Med Virol 94: 1540-1549, 2022.
29. Desai HD, Sharma K, Parikh A, Patel K, Trivedi J, Desai R, Patel PP, Patel Z, Patel S and Kini S: Predictors of mortality amongst tocilizumab administered COVID-19 asian indians: A predictive study from a tertiary care centre. Cureus 13: e13116, 2021.
30. Ercan S, Ergan B, Özuygur SS, Korkmaz P, Taşbakan MS, Basoglu ÖK, Kerget B, Akgün M, Elbek O, Sayıner A and Kılınç O: Clinical predictors of response to tocilizumab: A retrospective multicenter study. Turk Thorac J 23: 225-230, 2022.
31. Sarabia De Ardanaz L, Andreu-Ubero JM, Navidad-Fuentes M, Ferrer-González MÁ, Ruíz Del Valle V, Salcedo-Bellido I, Barrios-Rodríguez R, Cáliz-Cáliz R and Requena P: Tocilizumab in COVID-19: Factors associated with mortality before and after treatment. Front Pharmacol 12: 620187, 2021.
32. Pagkratis K, Chrysikos S, Antonakis E, Pandi A, Kosti CN, Markatis E, Hillas G, Digalaki A, Koukidou S, Chaini E, *et al*: Predictors of mortality in tocilizumab-treated severe COVID-19. Vaccines (Basel) 10: 978, 2022.
33. Masotti L, Landini G, Panigada G, Grifoni E, Tarquini R, Cei F, Cimolato BMA, Vannucchi V, Di Pietro M, Piani F, *et al*: Predictors of poor outcome in tocilizumab treated patients with Sars-CoV-2 related severe respiratory failure: A multicentre real world study. Int Immunopharmacol 107: 108709, 2022.
34. Mussini C, Cozzi-Lepri A, Menozzi M, Meschiari M, Franceschini E, Milic J, Brugioni L, Pietrangelo A, Girardis M, Cossarizza A, *et al*: Development and validation of a prediction model for tocilizumab failure in hospitalized patients with SARS-CoV-2 infection. PLoS One 16: e0247275, 2021.
35. Biolè C, Bianco M, Núñez-Gil IJ, Cerrato E, Spirito A, Roubin SR, Viana-Llamas MC, Gonzalez A, Castro-Mejía AF, Eid CM, *et al*: Gender differences in the presentation and outcomes of hospitalized patients with COVID-19. J Hosp Med 16: 349-352, 2021.
36. Brook R, Lim HY, Ho P and Choy KW: Risk factors and early prediction of clinical deterioration and mortality in adult COVID-19 inpatients: An Australian tertiary hospital experience. Intern Med J 52: 550-558, 2022.
37. Fortunato F, Martinelli D, Lo Caputo S, Santantonio T, Dattoli V, Lopalco PL and Prato R: Sex and gender differences in COVID-19: An Italian local register-based study. BMJ Open 11: e051506, 2021.
38. Georgakopoulou VE, Garmpis N, Damaskos C, Valsami S, Dimitroulis D, Diamantis E, Farmaki P, Papageorgiou CV, Makrodimitri S, Gravvanis N, *et al*: The impact of peripheral eosinophil counts and eosinophil to lymphocyte ratio (ELR) in the clinical course of COVID-19 patients: A retrospective study. In Vivo 35: 641-648, 2021.
39. Georgakopoulou VE, Lembessis P, Skarlis C, Gkoufa A, Sipsas NV and Mavragani CP: Hematological abnormalities in COVID-19 disease: Association with type I interferon pathway activation and disease outcomes. Front Med (Lausanne) 9: 850472, 2022.
40. Georgakopoulou VE, Vlachogiannis NI, Basoulis D, Eliadi I, Georgiopoulos G, Karamanakos G, Makrodimitri S, Samara S, Triantafyllou M, Voutsinas PM, *et al*: A simple prognostic score for critical COVID-19 derived from patients without comorbidities performs well in unselected patients. J Clin Med 11: 1810, 2022.
41. Georgakopoulou VE, Makrodimitri S, Triantafyllou M, Samara S, Voutsinas PM, Anastasopoulou A, Papageorgiou CV, Spandidos DA, Gkoufa A, Papalexis P, *et al*: Immature granulocytes: Innovative biomarker for SARS-CoV-2 infection. Mol Med Rep 26: 217, 2022.
42. Cholongitas E, Bali T, Georgakopoulou VE, Giannakodimos A, Gyftopoulos A, Georgilaki V, Gerogiannis D, Basoulis D, Eliadi I, Karamanakos G, *et al*: Prevalence of abnormal liver biochemistry and its impact on COVID-19 patients' outcomes: A single-center Greek study. Ann Gastroenterol 35: 290-296, 2022.
43. Tom J, Bao M, Tsai L, Qamra A, Summers D, Carrasco-Triguero M, McBride J, Rosenberger CM, Lin CJF, Stubbings W, *et al*: Prognostic and predictive biomarkers in patients with coronavirus disease 2019 treated with tocilizumab in a randomized controlled trial. Crit Care Med 50: 398-409, 2022.
44. Lohse A, Klopfenstein T, Balblanc JC, Royer PY, Bossert M, Gendrin V, Charpentier A, Bozgan AM, Badie J, Bourgoin C, *et al*: Predictive factors of mortality in patients treated with tocilizumab for acute respiratory distress syndrome related to coronavirus disease 2019 (COVID-19). Microbes Infect 22: 500-503, 2020.
45. Lakatos B, Szabo BG, Bobek I, Gopcsa L, Beko G, Kiss-Dala N, Petrik B, Gaspar Z, Farkas BF, Sinko J, *et al*: Laboratory parameters predicting mortality of adult in-patients with COVID-19 associated cytokine release syndrome treated with high-dose tocilizumab. Acta Microbiol Immunol Hung: Aug 6, 2021 (Epub ahead of print).
46. Bone RC, Francis PB and Pierce AK: Intravascular coagulation associated with the adult respiratory distress syndrome. Am J Med 61: 585-589, 1976.
47. Peiris JS, Yuen KY, Osterhaus AD and Stöhr K: The severe acute respiratory syndrome. N Engl J Med 349: 2431-2441, 2003.
48. Liu Y, Sun W, Guo Y, Chen L, Zhang L, Zhao S, Long D and Yu L: Association between platelet parameters and mortality in coronavirus disease 2019: Retrospective cohort study. Platelets 31: 490-496, 2020.