



Th1/Th2/Th17 Cytokines as Mortality Predictors in Older Adults With COVID-19 Pneumonia

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Abstract

Background: Dysregulated cytokine responses play a critical role in the pathogenesis of COVID-19; however, the age-specific dynamics of Th1/Th2/Th17 responses and their interactions with bacterial co-infections in older adults with COVID-19 pneumonia remain poorly understood.

Objectives: This study examined Th1/Th2/Th17 cytokine profiles (IL-2, IL-4, IL-6, IL-10, TNF- α , IFN- γ , IL-17A) in hospitalized older adults with COVID-19 pneumonia, assessed their association with mortality, and further evaluated their independent prognostic value.

Methods: A total of 231 hospitalized patients aged 60 years and older were enrolled in this retrospective study. Retrospective data were collected to assess age-related differences, disease progression, the impact of bacterial co-infections, and mortality rates. Stepwise logistic regression was used to identify key confounding factors, and multivariable models were used to evaluate independent prognostic indicators.

Results: IL-6 levels were significantly higher in the 80 - 89 age group than in the 60 - 69 and 70 - 79 age groups ($P = 0.002$ and $P = 0.009$). IL-6 peaked within the first three days after symptom onset and remained elevated throughout the course of the illness. IL-10 levels were higher during days 1 - 9 than during days 10 - 18 ($P = 0.006$). SARS-CoV-2 RNA-positive (S-RNA+) patients had higher IL-6 and IL-10 levels than RNA-negative (S-RNA-) cases ($P = 0.007$, $P = 0.03$). Among S-RNA+ patients, those with bacterial co-infection (S-RNA+BI) showed further elevations in IL-6 ($P = 0.03$). The three groups showed significant differences in mortality ($P < 0.001$), with the highest rate in the S-RNA+BI group. In non-survivors, elevated IL-4, IL-6, and IL-10 were observed in the S-RNA+BI subgroup, whereas only IL-6 and IL-10 were elevated in the S-RNA+ subgroup. ROC analysis identified the optimal cut-off values of these cytokines for predicting mortality. After adjustment for CURB-65 in logistic regression, only IL-6 independently predicted mortality, whereas IL-4 and IL-10 were only associated with disease severity.

Conclusions: Univariate analysis indicated that IL-4, IL-6, and IL-10 were correlated with mortality in older adult patients with COVID-19 pneumonia, particularly those with bacterial co-infections. After adjustment for disease severity, only IL-6 retained independent prognostic value for mortality.

Keywords: Coronavirus Disease 2019 (COVID-19), Coronavirus, Dialysis, Financial Burden, Safety, Immune System, Stroke, Mortality, Bacterial Co-infection, Sense Of Belonging, Social Support, Neighborhood, Older Adults

1. Background

The COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, remains a major public health challenge worldwide (1). Its impact has been particularly severe in vulnerable populations, especially individuals aged 60 years and older (2). In older adults, SARS-CoV-2

infection often results in more severe complications (3). Mortality rates among older adults with COVID-19 pneumonia are also markedly higher (4). This increased risk is likely attributable, at least in part, to age-related physiological changes that weaken immune function and exacerbate infection-induced inflammation (5).

Cytokines, proteins or peptides integral to the immune response, have been implicated in the severity of COVID-19 (6). Pro-inflammatory cytokines, such as interleukin (IL)-2, IL-6, tumor necrosis factor-alpha (TNF- α), and interferon-gamma (IFN- γ), are essential for initiating immune responses to infection (7). In contrast, anti-inflammatory cytokines, such as IL-10, regulate inflammation and maintain immune homeostasis during prolonged infections (8). Previous studies have highlighted the involvement of multiple cytokines, including IL-2, IL-4, IL-6, IL-10, TNF- α , IFN- γ , and IL-17A, in the pathological process of COVID-19 (6, 9-11). Notably, serum IL-6 levels have been reported to be significantly higher in severe than in non-severe COVID-19 cases (6, 12). However, levels of other cytokines, such as IL-10, TNF- α , and IFN- γ , vary between severe and non-severe cases, with some studies reporting elevations and others reporting no such increases (12, 13). This variability may reflect differences in the age distribution of the patient populations studied.

Mean IL-2, IL-6, IL-10, and TNF- α levels in patients with COVID-19 have been shown to correlate positively with advancing age (6). The review by Witkowski underscores that immunosenescence and inflammaging contribute to heterogeneous immune responses in older adults (5). Despite the central role of cytokine networks in inflammaging, their age-stratified dynamics in COVID-19 remain insufficiently characterized. A clearer understanding of cytokine-mediated immune dysregulation in older adults with COVID-19 pneumonia is essential for improving clinical management and patient outcomes.

2. Objectives

Given this context, the present study aimed to investigate cytokine profiles in older adults with COVID-19 pneumonia, with a focus on their associations with age, disease progression, comorbid bacterial infections, and mortality outcomes. Our data suggest that IL-4, IL-6, and IL-10 may help identify high-risk cases, particularly when their levels exceed specific thresholds. These biomarkers may assist clinicians in identifying patients who require aggressive intervention before clinical deterioration.

3. Methods

3.1. Study Population

This retrospective study was conducted at the Affiliated Wuxi People's Hospital of Nanjing Medical University. The source population comprised all

consecutive inpatients aged 60 years or older who were admitted to the Department of Respiratory and Critical Care Medicine between December 2022 and August 2023. The inclusion criteria were as follows: 1) age of 60 years or older; 2) diagnosis of COVID-19 pneumonia according to the Clinical Protocol for the Treatment of Novel Coronavirus Infection (Trial Version 10); and 3) availability of complete demographic, clinical, laboratory, and outcome data. The exclusion criteria were as follows: 1) missing Th1/Th2/Th17 cytokine measurements; 2) incomplete medical records; and 3) transfer to another hospital against medical advice. A total of 231 older adults with COVID-19 pneumonia were ultimately enrolled. All enrolled patients met the inclusion criteria, and no individuals were excluded based on the exclusion criteria during enrollment.

All patients had completed primary vaccination under the national vaccination campaign. Medical records were retrospectively reviewed to collect demographic and clinical information, including sex, age, clinical diagnosis, disease progression, CURB-65 score, and outcomes. The time points of symptom onset, hospital admission, SARS-CoV-2 nucleic acid testing, and discharge outcomes were systematically documented for all participants. Th1/Th2/Th17 cytokine levels were measured in peripheral blood samples collected at admission, and only one measurement per patient was included in the analysis. Of the 231 patients, 168 (72.73%) were sampled within 24 hours of admission, 22 (9.52%) within 24 - 48 hours, and the remaining 41 (17.75%) beyond 48 hours. Other laboratory data included SARS-CoV-2 RNA results, complete blood count (CBC), C-reactive protein (CRP), procalcitonin (PCT), and sputum culture results. Antiviral treatment with Paxlovid and antibacterial therapy were administered according to clinical conditions and microbiological results. The demographic and clinical data of older adults with COVID-19 pneumonia are presented in [Table 1](#).

This study was approved by the Ethics Committee of the Affiliated Wuxi People's Hospital of Nanjing Medical University (permit number: KY25029), and all procedures adhered to the ethical standards of the 1975 Declaration of Helsinki.

3.2. Grouping

Patients were stratified into three groups according to SARS-CoV-2 RNA status at admission and bacterial co-infection: 1) SARS-CoV-2 RNA-positive without bacterial co-infection (S-RNA+); 2) SARS-CoV-2 RNA-positive with bacterial co-infection (S-RNA+BI); and 3) SARS-CoV-2 RNA-negative (S-RNA-). All S-RNA- patients had negative SARS-CoV-2 polymerase chain reaction (PCR) tests (2019-

Table 1. Demographic and Clinical Data of Older Adults with COVID-19 Pneumonia ^a

Groups	Positive		Negative		Coinfection	
	Improved	Death	Improved	Death	Improved	Death
Age (y)	77.44 ± 8.94	85.20 ± 7.46	75.67 ± 7.45	68	79.95 ± 8.59	82.13 ± 7.11
Male	76	3	15	-	40	14
Female	36	2	21	1	22	1
Time to admission (d)	9.60 ± 5.89	9.20 ± 6.22	10.00 ± 6.94	10	10.37 ± 5.54	11.67 ± 8.47
Hospital days	10.24 ± 7.14	12.60 ± 6.27	6.97 ± 2.81	21	11.61 ± 7.40	14.80 ± 10.63
Chronic underlying disease						
Hypertension	62 (55.36)	1 (20.00)	27 (75.00)	1 (100)	30 (48.39)	5 (33.33)
Diabetes	31 (27.68)	2 (40.00)	13 (36.11)	0 (0)	14 (22.58)	1 (6.67)
Heart disease	25 (22.32)	1 (20.00)	4 (11.11)	0 (0)	18 (29.03)	3 (20.00)
Malignant tumor	12 (10.71)	1 (20.00)	1 (2.78)	0 (0)	11 (17.74)	2 (13.33)
Chronic lung disease	8 (7.14)	0 (0)	1 (2.78)	0 (0)	4 (6.45)	0 (0)
Cerebrovascular disease	4 (3.57)	0 (0)	2 (5.56)	0 (0)	8 (12.90)	0 (0)
Admission CURB-65 score	1.79 ± 0.70	2.40 ± 0.55	1.69 ± 0.67	2.0	2.10 ± 0.69	2.33 ± 0.62

^a Values are expressed as number, No. (%) or mean ± SD. Abbreviations: > COVID-19, coronavirus disease 2019; CURB-65, confusion, urea, respiratory rate, blood pressure, age ≥ 65 years.

nCoV Nucleic Acid Detection Kit, Sansure Biotech) at admission and a confirmed SARS-CoV-2 infection within the preceding 4 weeks; those with earlier infection were not included. Bacterial co-infection was defined by at least one of the following: positive sputum culture, white blood cell count (WBC) > 10 × 10⁹/L, CRP > 100 mg/L, or PCT > 0.5 ng/mL. Most patients received empirical or prophylactic antibiotics before or shortly after admission, reducing the sputum culture positivity rate. Given this limitation, we used a panel of laboratory indicators to screen for potential bacterial co-infection in this retrospective cohort. Based on established clinical guidelines, abnormal WBC, CRP, and PCT values were combined to identify secondary bacterial infection. However, these inflammatory markers lack bacterial specificity. Severe COVID-19 itself can trigger robust systemic inflammation and increase these indicators, potentially resulting in false-positive classification of bacterial co-infection. In the S-RNA+BI group, 51 patients were identified solely by inflammatory biomarkers, and 26 cases were confirmed by positive sputum culture.

To explore age-related differences in cytokine levels, the S-RNA+ group was further categorized into four age subgroups: 60 - 69, 70 - 79, 80 - 89, and 90 - 100 years. To assess changes in cytokine levels during disease progression, this group was also subdivided by the interval from disease onset to cytokine measurement (1 - 3, 4 - 6, 7 - 9, 10 - 12, 13 - 15, and 16 - 18 days), with subgroups containing fewer than 5 cases excluded.

3.3. Th1/Th2/Th17 Cytokines in Blood Samples

Blood levels of Th1/Th2/Th17 cytokines, including IL-4, IL-6, IL-10, TNF-α, IFN-γ, and IL-17A, were obtained from the Laboratory Information System (LIS). All cytokines were measured using the BD Cytometric Bead Array Human Th1/Th2/Th17 Cytokine Kit.

3.4. Statistical Analysis

GraphPad Prism 9.5.1 and SPSS 22.0 were used for statistical analyses. The normality of all continuous data was assessed. Across multiple groups, non-normally distributed continuous variables were compared using the Kruskal-Wallis test; pairwise post hoc comparisons were performed using Dunn's test when significance ($P < 0.05$) was observed. Receiver operating characteristic (ROC) curve analysis with the Youden index was used to calculate optimal cutoff values for IL-4, IL-6, and IL-10. Continuous variables were presented as mean ± standard deviation (SD). For between-group comparisons, non-normally distributed continuous variables were analyzed using the Mann-Whitney U test, whereas categorical variables were analyzed using the χ^2 test. Missing data were handled using complete-case analysis.

To identify independent predictors of in-hospital mortality, we performed stepwise logistic regression to screen confounders (entry: $P < 0.10$; removal: $P > 0.11$). Candidate covariates were selected based on clinical relevance and previous literature, including age, sex,

Table 2. Age-Specific Variations in Cytokine Profiles Among S-RNA+ Group Patients ^a

Ages	IL-2 ^b	IL-4 ^b	IL-6 ^b	IL-10 ^b	TNF- α ^b	IFN- γ ^b	IL-17A ^b
60 - 69 (n = 24)	1.96 $\pm$ 1.66	1.60 $\pm$ 1.26	6.08 $\pm$ 5.16 ^c	12.63 $\pm$ 33.54	1.88 $\pm$ 1.09	2.35 $\pm$ 1.70	4.00 $\pm$ 2.76
70 - 79 (n = 43)	2.05 $\pm$ 1.97	1.88 $\pm$ 1.38	12.50 $\pm$ 18.69 ^d	5.55 $\pm$ 4.45	1.98 $\pm$ 0.81	2.46 $\pm$ 1.66	5.02 $\pm$ 5.50
80 - 89 (n = 39)	1.80 $\pm$ 1.36	1.84 $\pm$ 1.09	35.86 $\pm$ 42.12	5.55 $\pm$ 7.39	1.80 $\pm$ 0.75	2.10 $\pm$ 1.12	4.96 $\pm$ 4.93
90 - 100 (n = 11)	0.89 $\pm$ 0.74	1.35 $\pm$ 1.01	17.86 $\pm$ 16.75	3.35 $\pm$ 1.78	1.76 $\pm$ 0.74	1.62 $\pm$ 0.72	5.97 $\pm$ 6.39

^a Values are expressed as mean $\pm$ SD. Abbreviations: S-RNA+, patients positive for SARS-CoV-2 RNA without bacterial co-infection.

^b The serum concentration unit is pg/mL.

^c P = 0.002, 60 - 69 versus 80 - 89 age group.

^d P = 0.009, 70 - 79 versus 80 - 89 age group.

hypertension, diabetes, heart disease, malignancy, chronic respiratory disease, cerebrovascular disease, and baseline CURB-65 score. After screening, multivariable logistic regression was used to assess the independent association between cytokines and mortality after adjustment for major confounders. All tests were two-sided, and P < 0.05 indicated statistical significance.

4. Results

4.1. Cytokine Levels in Older Adults With COVID-19 Pneumonia Across Age Groups

This study included 231 older adults with COVID-19 pneumonia, stratified into four age groups: 60 - 69, 70 - 79, 80 - 89, and 90 - 100 years. IL-6 levels gradually increased with age in the 60 - 69, 70 - 79, and 80 - 89 groups. The 80 - 89-year group had significantly higher IL-6 levels (35.86 $\pm$ 42.12 pg/mL) than the 60 - 69-year group (6.08 $\pm$ 5.16 pg/mL; P = 0.002) and the 70 - 79-year group (12.50 $\pm$ 18.69 pg/mL; P = 0.009). By contrast, IL-6 levels did not increase further in the 90 - 100-year group. No significant intergroup differences were observed for IL-2, IL-4, IL-10, TNF- α , IFN- γ , or IL-17A across age subgroups (Table 2).

4.2. Cytokine Levels in Older Adults With COVID-19 Pneumonia Across Different Disease Courses

This study analyzed cytokine levels in S-RNA+ patients with COVID-19 pneumonia and their association with disease progression. Serum IL-6 peaked within the first 3 days of illness and then decreased significantly (Table 1 in Supplementary File), although it remained elevated above the normal reference range (> 5.30 pg/mL). No statistically significant differences in IL-6 levels were detected across time points during the disease course.

Serum IL-10 levels varied. They exceeded the reference value (4.91 pg/mL) at 1 - 3, 4 - 6, and 7 - 9 days but fell below it at 10 - 12, 13 - 15, and 16 - 18 days, with no significant differences across these time points. When grouped into 1 - 9 days and 10 - 18 days, IL-10 levels were significantly higher in the early period (6.70 $\pm$ 6.35 vs 3.54 $\pm$ 1.81; U = 852.5; P = 0.006). In contrast, serum concentrations of IL-2, IL-4, TNF- α , IFN- γ , and IL-17A remained below the reference value at all time points, with no significant between-group differences.

4.3. Cytokine Levels in Older Adults With COVID-19 Pneumonia in the S-RNA+, S-RNA-, and S-RNA+BI Groups

We also compared serum cytokine levels among older adults with COVID-19 pneumonia in the S-RNA+, S-RNA-, and S-RNA+BI groups (Table 3). Serum IL-6 levels were significantly higher in the S-RNA+ group than in the S-RNA- group (Z = 2.94; P = 0.007) and were further elevated in the S-RNA+BI group (Z = 2.40; P = 0.03). Moreover, serum IL-10 levels were higher in the S-RNA+ group than in the S-RNA- group (Z = 2.44; P = 0.03).

4.4. Analysis of Cytokine Markers for Predicting Mortality Events

We investigated the association between mortality and cytokine levels across groups. Mortality differed significantly among the three groups (χ^2 = 18.82; P < 0.001), with the S-RNA+BI group showing a markedly higher mortality rate (19.48%) than the S-RNA+ group (4.27%) and the S-RNA- group (2.70%). As presented in Table 4, non-survivors in the S-RNA+BI group had significantly higher serum IL-4, IL-6, and IL-10 levels than survivors (P = 0.04, P < 0.001, and P = 0.001, respectively). Likewise, non-survivors in the S-RNA+ group had significantly higher IL-6 and IL-10 levels than survivors (P = 0.002 and P = 0.02, respectively).

Table 3. Cytokine Levels in S-RNA+, S-RNA-, and S-RNA+BI COVID-19 Pneumonia Cohorts

Groups	IL-2 ^a	IL-4 ^a	IL-6 ^a	IL-10 ^a	TNF- α ^a	IFN- γ ^a	IL-17A ^a
S-RNA+ (n = 117)	1.84 $\pm$ 1.64	1.76 $\pm$ 1.23	19.20 $\pm$ 29.71	6.79 $\pm$ 16.05	1.88 $\pm$ 0.85	2.24 $\pm$ 1.45	4.88 $\pm$ 4.92
S-RNA- (n = 37)	1.38 $\pm$ 1.25	2.05 $\pm$ 1.72	8.11 $\pm$ 14.86 ^b	4.29 $\pm$ 6.73 ^d	1.93 $\pm$ 0.98	2.00 $\pm$ 1.14	5.93 $\pm$ 4.33
S-RNA+BI (n = 77)	1.90 $\pm$ 2.03	2.04 $\pm$ 1.73	70.75 $\pm$ 202.20 ^c	7.78 $\pm$ 15.02	2.09 $\pm$ 1.13	4.72 $\pm$ 14.64	6.97 $\pm$ 6.72

^aValues are expressed as mean $\pm$ SD. The serum concentration unit is pg/mL.

^bP = 0.007, SARS-CoV-2 RNA-positive (S-RNA+) versus SARS-CoV-2 RNA-negative (S-RNA-) group.

^cP = 0.03, S-RNA+ versus SARS-CoV-2 RNA-positive with bacterial infection (S-RNA+BI) group.

^dP = 0.03, S-RNA+ versus S-RNA- group.

To identify potential cytokine markers for mortality prediction, ROC analysis was performed in the S-RNA+ and S-RNA+BI groups to determine optimal cutoff values using the Youden index. In the S-RNA+ group (Figure 1A), IL-6 showed the highest area under the curve (AUC) (0.88; 95% CI, 0.76 - 1.00; P = 0.004; sensitivity, 100%; specificity, 69.64%), followed by IL-10 (0.80; 95% CI, 0.65 - 0.95; P = 0.02; sensitivity, 100%; specificity, 58.93%). The optimal cutoffs for predicting mortality risk were IL-6 > 12.20 pg/mL and IL-10 > 4.43 pg/mL. In the S-RNA+BI group (Figure 1B), IL-6 again had the highest AUC (0.87; 95% CI, 0.77 - 0.97; P < 0.001; sensitivity, 66.67%; specificity, 69.64%), followed by IL-10 (0.77; 95% CI, 0.64 - 0.89; P = 0.002; sensitivity, 66.67%; specificity, 80.65%) and IL-4 (0.67; 95% CI, 0.53 - 0.82; P = 0.04; sensitivity, 66.67%; specificity, 66.13%). The corresponding optimal cutoffs were IL-4 > 1.91 pg/mL, IL-6 > 65.45 pg/mL, and IL-10 > 6.35 pg/mL.

4.5. Adjusted Regression Analysis of Cytokines and Mortality

After stepwise multivariate regression, the baseline CURB-65 score was confirmed as a key independent factor for mortality (B = 0.079; SE = 0.026; β = 0.194; t = 2.997; P = 0.003). We further adjusted regression models for CURB-65 to assess the independent prognostic roles of IL-4, IL-6, and IL-10. After adjustment, higher IL-6 remained significantly associated with mortality and acted as an independent prognostic marker (OR = 1.025; 95% CI, 1.013 - 1.037; P < 0.001). By comparison, IL-4 and IL-10 showed no significant correlation with mortality after correction, suggesting that their prognostic relevance depended on overall disease severity.

5. Discussion

This study provides a detailed characterization of Th1/Th2/Th17 cytokine profiles in older adults hospitalized with COVID-19 pneumonia. It identifies age-related patterns, disease progression dynamics, and associations with bacterial co-infection and mortality.

Our findings indicate that IL-4, IL-6, and IL-10 are key immunological indicators reflecting clinical severity and may serve as early predictors of poor outcomes.

We observed a progressive increase in IL-6 levels with age up to the 80 - 89-year group, followed by a plateau in nonagenarians. This pattern aligns with the concept of inflammaging, in which chronic low-grade inflammation escalates with age, whereas immune responsiveness becomes blunted in extreme old age (14, 15). The early peak in IL-6 during the first 3 days of illness indicates a hyperinflammatory response at disease onset. IL-6 remained elevated throughout the disease course, underscoring a persistent inflammatory state in older adults with COVID-19 pneumonia (16). In contrast, IL-10 showed an early, transient increase during days 1 - 9, likely reflecting a compensatory anti-inflammatory response that counterbalances IL-6-mediated hyperinflammation. These temporal dynamics provide a rationale for the timing of anti-cytokine therapies, such as IL-6 receptor blockade, during the early hyperinflammatory phase.

A comparison of cytokine levels across the S-RNA+, S-RNA-, and S-RNA+BI groups showed that bacterial co-infection amplifies systemic inflammation, as evidenced by stepwise increases in IL-6 and IL-10 in the S-RNA+BI cohort. The markedly higher IL-6 levels in these patients support earlier findings that co-infections can intensify inflammatory responses and lead to worse clinical outcomes (17). Notably, we identified IL-4 as an additional mortality-associated cytokine in the S-RNA+BI group. This novel finding suggests that Th2-mediated responses may contribute to fatal outcomes when bacterial co-infections coexist with SARS-CoV-2 infection.

Mortality analyses showed that increased IL-6 and IL-10 levels were associated with mortality across both S-RNA+ cohorts. After stepwise multivariate regression, the baseline CURB-65 score was confirmed as a key independent factor for mortality. We therefore adjusted subsequent regression models for CURB-65 to assess the

Table 4. Cytokine Levels in Survivors and Non-Survivors in COVID-19 Pneumonia Cohorts^a

Groups and Outcomes	IL-2 ^b	IL-4 ^b	IL-6 ^b	IL-10 ^b	TNF- α ^b	IFN- γ ^b	IL-17A ^b
S-RNA+							
Survivors (n = 112)	1.84 $\pm$ 1.66	1.75 $\pm$ 1.24	16.85 $\pm$ 24.13	6.42 $\pm$ 15.85	1.87 $\pm$ 0.85	2.20 $\pm$ 1.45	4.88 $\pm$ 4.84
Non-survivors (n = 5)	1.79 $\pm$ 0.93	1.89 $\pm$ 1.00	78.39 $\pm$ 69.00 ^d	15.14 $\pm$ 18.73 ^f	2.12 $\pm$ 0.78	3.10 $\pm$ 0.76	4.99 $\pm$ 6.74
S-RNA+BI							
Survivors (n = 62)	1.79 $\pm$ 1.49	1.90 $\pm$ 1.75	20.50 $\pm$ 33.30	7.14 $\pm$ 16.43	2.04 $\pm$ 1.21	3.66 $\pm$ 10.70	6.40 $\pm$ 6.48
Non-survivors (n = 15)	2.38 $\pm$ 3.51	2.63 $\pm$ 1.55 ^c	278.46 $\pm$ 399.00 ^e	10.44 $\pm$ 6.23 ^g	2.25 $\pm$ 0.75	9.11 $\pm$ 25.29	9.29 $\pm$ 7.40

^aValues are expressed as mean $\pm$ SD. Abbreviations: S-RNA+, SARS-CoV-2 RNA-positive; S-RNA+BI, SARS-CoV-2 RNA-positive with bacterial infection.

^bThe serum concentration unit is pg/mL.

^cP = 0.04 survivors versus non-survivors in the S-RNA+BI group.

^dP < 0.001 survivors versus non-survivors in the S-RNA+ group.

^eP = 0.002 survivors versus non-survivors in the S-RNA+BI group.

^fP = 0.02, survivors versus non-survivors in the S-RNA+ group.

^gP = 0.001, survivors versus non-survivors in the S-RNA+BI group.

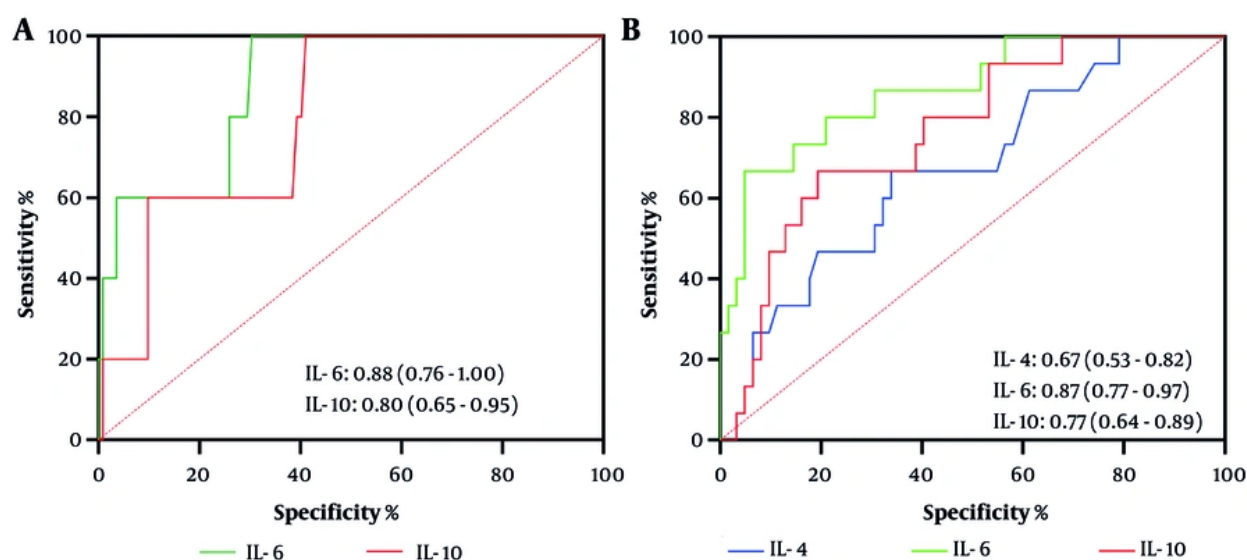


Figure 1. Receiver operating characteristic (ROC) curve analysis was conducted to distinguish survivors from non-survivors in the S-RNA+ (112 versus 5) and S-RNA+BI (62 versus 15) groups. An area under the ROC curve (AUC) closer to 1 indicates higher diagnostic accuracy. AUC data are presented as values (95% confidence intervals). A, In the S-RNA+ group, IL-6 showed the highest AUC value, sensitivity, and specificity. B, In the S-RNA+BI group, IL-6 showed the highest AUC value, while IL-10 had the highest specificity. The cutoff values are internally derived and exploratory unless externally validated.

independent prognostic roles of IL-4, IL-6, and IL-10. After adjustment, only higher IL-6 remained significantly associated with mortality, serving as an independent prognostic marker. Neither IL-4 nor IL-10 retained significance after correction, indicating that their crude associations with mortality were largely driven by underlying disease severity.

Despite the lack of independent prognostic value after CURB-65 adjustment, IL-10 and IL-4 were included in ROC analyses based on their univariate associations to derive optimal cutoff values. In S-RNA+ patients, IL-6 > 12.2 pg/mL and IL-10 > 4.43 pg/mL showed high sensitivity for mortality prediction; in the S-RNA+BI group, IL-6 > 65.45 pg/mL, IL-10 > 6.35 pg/mL, and IL-4 > 1.91 pg/mL identified high-risk patients. Although only

IL-6 was an independent prognostic biomarker, evaluation of IL-4 and IL-10 alongside IL-6 provides supplementary prognostic value, supporting a refined biomarker-assisted strategy for clinical risk stratification.

Interestingly, TNF- α , IFN- γ , IL-2, and IL-17A remained largely unchanged across age groups and disease stages (13). This suggests that the cytokine-driven pathophysiological response in older adults with COVID-19 pneumonia is dominated by IL-6, IL-10, and IL-4 (18, 19). It also highlights the importance of age-tailored immunomodulatory strategies rather than broad targeting of multiple cytokines.

5.1. Limitations

This study has several limitations. First, its single-center retrospective design may introduce selection bias and limit generalizability. Second, the relatively small sample size of the nonagenarian subgroup warrants cautious interpretation of the corresponding results. Third, causality cannot be established from observational data. Fourth, the small number of mortality events in some subgroups may reduce the reliability of ROC analyses and increase the risk of overfitting. Fifth, all derived cytokine cutoff values are exploratory internal findings and have not been validated in an external cohort.

5.2. Conclusions

This study identifies distinct cytokine signatures in older adults with COVID-19 pneumonia, highlighting IL-6, IL-10, and IL-4 as clinically relevant immune indicators for evaluating clinical severity and mortality risk. These findings provide a basis for early risk stratification and targeted interventions, especially in patients with bacterial co-infections. Large-scale, prospective, multicenter investigations are therefore needed to validate the clinical utility of these cytokine cutoffs and further refine risk stratification and targeted therapy in this vulnerable population.

Supplementary Material

Supplementary material(s) is available [here](#) [To read supplementary materials, please refer to the journal website and open PDF/HTML].

Footnotes

AI Use Disclosure: The authors declare that no generative AI tools were used in the creation of this

article.

Authors' Contribution: H. M. and H. Z. contributed to the study concept and design and provided administrative, technical, and material support. H. M. and Y. Y. contributed to data acquisition and data analysis and interpretation. H. M. drafted the manuscript and performed the statistical analysis. H. Z. critically revised the manuscript for important intellectual content and supervised the study.

Conflict of Interests Statement: The authors do not declare any conflicts of interests for this study.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication. The data are not publicly available due to privacy and ethical restrictions regarding participant information.

Ethical Approval: This study was approved by the Ethics Committee of the Affiliated Wuxi People's Hospital of Nanjing Medical University (Permit number: KY25029).

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Informed Consent: Informed consent was obtained from all participants.

References

1. Watson OJ, Barnsley G, Toor J, Hogan AB, Winskill P, Ghani AC. Global impact of the first year of COVID-19 vaccination: a mathematical modelling study. *Lancet Infect Dis.* 2022;**22**(9):1293-1302. [PubMed ID: 35753318]. [PubMed Central ID: PMC9225255]. [https://doi.org/10.1016/S1473-3099\(22\)00320-6](https://doi.org/10.1016/S1473-3099(22)00320-6).
2. Weng C, Xie R, Han G, Yuan Y, Li S, Wang C, et al. Safety and Efficacy of Paxlovid Against Omicron Variants of Coronavirus Disease 2019 in Elderly Patients. *Infect Dis Ther.* 2023;**12**(2):649-662. [PubMed ID: 36696068]. [PubMed Central ID: PMC9875765]. <https://doi.org/10.1007/s40121-023-00760-x>.
3. Xing Y, Li Y, Feng L, Huo R, Ma X, Dong Y, et al. Predictors of COVID-19 Severity in Elderly Patients Infected by Omicron in China, 18 December 2022 - 5 February 2023. *Infect Drug Resist.* 2023;**16**:4505-18. [PubMed ID: 37457796]. [PubMed Central ID: PMC10349581]. <https://doi.org/10.2147/IDR.S418622>.
4. O'Driscoll M, Ribeiro Dos Santos G, Wang L, Cummings DAT, Azman AS, Paireau J, et al. Age-specific mortality and immunity patterns of SARS-CoV-2. *Nature.* 2021;**590**(7844):140-145. [PubMed ID: 33137809]. <https://doi.org/10.1038/s41586-020-2918-0>.
5. Witkowski JM. Immune system aging and the aging-related diseases in the COVID-19 era. *Immunol Lett.* 2022;**243**:19-27. [PubMed ID: 35108570]. [PubMed Central ID: PMC8801734]. <https://doi.org/10.1016/j.imlet.2022.01.005>.
6. Islam F, Habib S, Badruddza K, Rahman M, Islam MR, Sultana S, et al. The Association of Cytokines IL-2, IL-6, TNF-alpha, IFN-gamma, and IL-10 With the Disease Severity of COVID-19: A Study From Bangladesh.

- Cureus*. 2024;**16**(4):e57610. [PubMed ID: 38707035]. [PubMed Central ID: PMC11069400]. <https://doi.org/10.7759/cureus.57610>.
7. Coperchini F, Chiovato L, Croce L, Magri F, Rotondi M. The cytokine storm in COVID-19: An overview of the involvement of the chemokine/chemokine-receptor system. *Cytokine Growth Factor Rev*. 2020;**53**:25-32. [PubMed ID: 32446778]. [PubMed Central ID: PMC7211650]. <https://doi.org/10.1016/j.cytogfr.2020.05.003>.
 8. Channappanavar R, Perlman S. Age-related susceptibility to coronavirus infections: role of impaired and dysregulated host immunity. *J Clin Invest*. 2020;**130**(12):6204-6213. [PubMed ID: 33085654]. [PubMed Central ID: PMC7685716]. <https://doi.org/10.1172/JCI144115>.
 9. Karasahin EF, Sebin E, Akin Sen I, Karaşahin O. The Value of Interleukin-17A as a Prognostic Indicator in COVID-19 Patients. *Jundishapur J Microbiol*. 2022;**15**(11). <https://doi.org/10.5812/ijjm-130316>.
 10. Fawzy S, Ahmed MM, Alsayed BA, Mir R, Amle D. IL-2 and IL-1beta Patient Immune Responses Are Critical Factors in SARS-CoV-2 Infection Outcomes. *J Pers Med*. 2022;**12**(10):1729. [PubMed ID: 36294868]. [PubMed Central ID: PMC9605386]. <https://doi.org/10.3390/jpm12101729>.
 11. Lu Q, Zhu Z, Tan C, Zhou H, Hu Y, Shen G, et al. Changes of serum IL-10, IL-1beta, IL-6, MCP-1, TNF-alpha, IP-10 and IL-4 in COVID-19 patients. *Int J Clin Pract*. 2021;**75**(9):e14462. [PubMed ID: 34107113]. [PubMed Central ID: PMC8237069]. <https://doi.org/10.1111/ijcp.14462>.
 12. Gupta G, Shareef I, Tomar S, Kumar MSN, Pandey S, Sarda R, et al. Th1/Th2/Th17 Cytokine Profile among Different Stages of COVID-19 Infection. *Natl Acad Sci Lett*. 2022;**45**(4):363-9. [PubMed ID: 35528283]. [PubMed Central ID: PMC9065657]. <https://doi.org/10.1007/s40009-022-01123-9>.
 13. Luo W, Zhang J, Zhang W, Lin Y, Wang Q. Circulating levels of IL-2, IL-4, TNF-alpha, IFN-gamma, and C-reactive protein are not associated with severity of COVID-19 symptoms. *J Med Virol*. 2021;**93**(1):89-91. [PubMed ID: 32519779]. [PubMed Central ID: PMC7300996]. <https://doi.org/10.1002/jmv.26156>.
 14. Alnakhli A, Albaloshi J, Cham LB. Aging and Infection: Impact of Immunosenescence and Inflammaging in Respiratory Viral Infections. *Cell Physiol Biochem*. 2026;**60**(1):73-88. [PubMed ID: 41808551]. <https://doi.org/10.33594/000000848>.
 15. Müller L, Di Benedetto S. Immunosenescence and inflammaging: Mechanisms and modulation through diet and lifestyle. *Front Immunol*. 2025;**16**. 1708280. [PubMed ID: 41425546]. [PubMed Central ID: PMC12711513]. <https://doi.org/10.3389/fimmu.2025.1708280>.
 16. Xu J, Zheng J, Tan Y, Cai J, Xiang Y, Ling H, et al. Longitudinal Observation of Immune Response for 23 Months in COVID-19 Convalescent Patients After Infection and Vaccination. *Viral Immunol*. 2023;**36**(6):389-400. [PubMed ID: 37276049]. <https://doi.org/10.1089/vim.2022.0111>.
 17. Liu S, Yu C, Tu Q, Zhang Q, Fu Z, Huang Y, et al. Bacterial co-infection in COVID-19: a call to stay vigilant. *PeerJ*. 2024;**12**. e18041. [PubMed ID: 39308818]. [PubMed Central ID: PMC11416760]. <https://doi.org/10.7717/peerj.18041>.
 18. Bi Q, Zhu J, Zheng J, Xu Q, Chen J, Zhang L, et al. Blood Inflammatory Markers and Cytokines in COVID-19 Patients With Bacterial Coinfections. *Immun Inflamm Dis*. 2024;**12**. e70105. [PubMed ID: 39692539]. [PubMed Central ID: PMC11653711]. <https://doi.org/10.1002/iid3.70105>.
 19. Jafrin S, Aziz MA, Islam MS. Elevated Levels of Pleiotropic Interleukin-6 (IL-6) and Interleukin-10 (IL-10) are Critically Involved With the Severity and Mortality of COVID-19: An Updated Longitudinal Meta-Analysis and Systematic Review on 147 Studies. *Biomark Insights*. 2022;**17**. 11772719221106600. [PubMed ID: 35747885]. [PubMed Central ID: PMC9209786]. <https://doi.org/10.1177/11772719221106600>.