Original Article

Comparative Analysis of Copeptin, IL-6, and TNF-α as Predictive Biomarkers for Clinical Outcome in Moderate to Severe COVID-19 Patients

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Abstract

Background: Cytokine release syndrome (CRS) plays a central role in disease progression, driven by pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), as well as neuroendocrine stress markers such as copeptin. Perbandingan langsung biomarker copeptin, IL-6, dan TNF-α sangat penting untuk mengidentifikasi prediktor hasil COVID-19 yang paling andal, terutama pada kasus sedang hingga berat. This retrospective clinical study aimed to evaluate the correlation of copeptin, IL-6, and TNF-α levels with the clinical outcomes of patients with moderate to severe COVID-19.

Methods: The study included patients who met the selection criteria and provided written informed consent. A sample size of 41 was determined via power analysis to achieve 80% power at a 0.05 significance level. Consecutive sampling was employed to select participants. The study utilized medical records of patients with moderate to severe COVID-19 who underwent copeptin, IL-6, and TNF-α testing. Correlation analyses and Bonferroni corrections were performed using SPSS® ver. 21.

Results: Results revealed a moderate positive correlation between copeptin levels and patient outcomes (Bonferroni correlation=0.597; *P*<0.001). A weak positive correlation was observed between IL-6 levels and outcomes (Bonferroni correlation=0.239; *P*=0.055), while a negligible positive correlation was found for TNF-α levels (Bonferroni correlation=0.140; *P*=0.177), which was not statistically significant.

Conclusion: Copeptin emerged as a more sensitive biomarker for predicting the outcomes of COVID-19 patients. Elevated copeptin levels were associated with a poorer prognosis. Furthermore, its stability in serum renders copeptin a more sensitive biomarker than IL-6 and TNF-α.

Keywords: copeptin, COVID-19, IL-6, SARS-CoV-2, TNF-α

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INTRODUCTION

The COVID-19 severity ranges from asymptomatic to life-threatening cases, such as acute respiratory distress syndrome (ARDS), which affects about 16% of hospitalized patients with severe pneumonia and markedly increases mortality.[1–3] Cytokine release syndrome (CRS), as a hyperinflammatory response, plays a central role in disease progression, driven by pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), as well as neuroendocrine stress markers such as copeptin.[4,5] TNF-α, produced by monocytes and macrophages, binds to TNFR1 to regulate cell death and immune cell recruitment, activates NF-κB, and promotes inflammation—processes that, when excessive, can worsen COVID-19 outcomes.[6,7]

The inflammatory response in COVID-19, including pain and stress, activates the hypothalamic-pituitary axis and triggers arginine vasopressin (AVP) secretion. Lung damage, hypoxia, and cytokines like IL-6 can further elevate AVP, impairing cardiac function.[6,8] This leads to elevated copeptin levels—a stable surrogate marker of AVP activity—which have been found to be significantly higher in severe COVID-19, indicating its prognostic potential.[6]

A direct comparison of the biomarkers copeptin, IL-6, and TNF-α is crucial for identifying the most reliable predictor of COVID-19 outcomes, particularly in moderate-to-severe cases. Furthermore, evidence regarding the role of

inflammatory markers in COVID-19 was growing, but research directly comparing copeptin, IL-6, and TNF-α remains limited. Therefore, this study aimed to compare these biomarkers to identify the most reliable predictor of outcomes in moderate to severe COVID-19 patients and to elucidate the role of TNF-α in modulating the host immune response to SARS-CoV-2 infection.

METHODS

This retrospective clinical study was conducted at Dr. Moewardi General Hospital, Surakarta, between October 2021 and March 2022, focusing on COVID-19 patients suspected of harboring the Omicron variant. The patient selection process was based on symptom severity, age, and confirmed COVID-19 diagnosis. Blood samples were collected and analyzed at the Clinical Pathology Laboratory of Dr. Moewardi General Hospital between the 5th and 7th days after symptom onset to capture the peak immune response.

Inclusion criteria for this study were adults (≥18 years) with confirmed moderate to severe COVID-19. Critically ill patients were directly categorized into the severe group. Moderate cases presented with pneumonia signs (fever, cough, dyspnea, respiratory rate ≥30/min) and SpO_2 >93%, while severe cases exhibited similar symptoms with SpO_2 <93% or severe respiratory distress.

Eleven patients with other infections or cytokine-altering conditions were excluded. A total of 41 patients (23 females, 18 males) met the criteria and provided consent. Consecutive sampling was applied, and power analysis confirmed that 41 participants provided 80% power at a 0.05 significance level.

Blood samples were separated into serum and plasma for analysis. The plasma samples were analyzed in the Clinical Pathology Laboratory of Dr. Moewardi General Hospital, and the tests were performed between the 5th and 7th days after symptom onset to ensure that immune responses were adequately represented. Nasopharyngeal swabs were collected from each participant and tested via PCR to confirm the diagnosis of COVID-19 and the presence of the Omicron variant. All blood samples were stored at -80°C until further analysis.

The copeptin levels were measured using the Human Copeptin ELISA Kit (ARG81384), with a normal reference range for copeptin being 1.70–11.25 pmol/L.12 IL-6 levels were assessed using the Demeditec Interleukin-6 Human ELISA (DE2132) kit, with a normal reference range for IL-6 being 0-43.5 pg/ml. TNF-α levels were determined using the Human TNF-α ELISA Kit (Invitrogen Cat #A35601), with a normal reference range for TNF-α of approximately 24.47 pg/mL.[6]

Data were collected from the hospital's medical records, and all patient data were anonymized for analysis. Statistical analysis was performed using SPSS® version 21. Descriptive statistics, including frequency distributions, means, and standard deviations, were used to summarize patient characteristics. Correlation analysis was performed using Pearson's or Spearman's tests as appropriate, with multiple comparisons adjusted using the Bonferroni correction to control for type I errors. Results were presented as narratives, frequency tables, and graphs describing the relationships between copeptin, IL-6, TNF-α, and clinical outcomes (mortality status). The significance threshold was set at $P<0.05$.

RESULTS

The characteristics of the research subjects are summarized in Table 1. The study population consisted of 23 females (56.1%) and 18 males (43.9%). The majority of patients were adults under 60 years old (73%), with the majority of severity classified as moderate in 63% of patients. Of the 41 patients, 25 (60.9%) survived, while 16 (39.1%) passed away during the treatment period. Hypertension was the most prevalent comorbidity, found in 39.1% of the cohort.

Figures 1, 2, and 3 show the distribution of copeptin, IL-6, and TNF-α levels in patients with COVID-19, with confirmed omicron infection. The values for each biomarker varied widely among

participants.

Table 1. Demographic and Clinical Characteristics of Research Subjects

Variable	n (%)
Age	
Adult (<60 years old)	30 (73.0%)
Elderly (≥60 years old)	11 (27.0%)
Gender	
Male	18 (43.9%)
Female	23 (56.1%)
Severity	
Moderate	26 (63.0%)
Severe	15 (37.0%)
Outcome	
Survived	25 (60.9%)
Died	16 (39.1%)
Comorbidity	
Diabetes Mellitus (DM)	10 (23.9%)
Hypertension	16 (39.1%)
Cardiovascular	0 (0.0%)
Chronic Acute Lung Disease	5 (13.0%)
Malignancy	5 (13.0%)
Liver Disorders	2 (4.3%)
Renal Disease	0 (0.0%)
Pregnancy	0 (0.0%)
Cerebrovascular	1 (2.2%)
Fracture	4 (10.9%)
HIV/AIDS	1 (2.2%)
Anemia	11 (26.1%)
Without Comorbid	8 (19.6%)

Copeptin levels ranged from 0.06 pmol/L (found in a 50-year-old male, moderate case) to 4.6 pmol/L (found in a 71-year-old male, critical case). IL-6 levels ranged from 2 pg/mL (found in a 40-year-old male, moderate case) to 456 pg/mL (found in a 61-year-old male, critical case), while TNF-α levels ranged from 3.43 pg/mL (found in a 44-year-old male) to 434.03 pg/mL (fpund in a 56-year-old female, critical case). Among 41 patients, 25 patients (60.9%) survived, and 16 patients (39.1%) died during treatment.

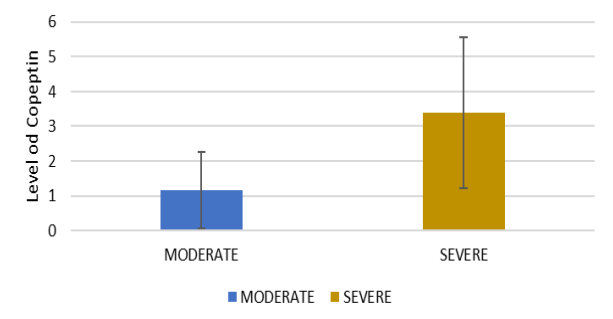

Figure 1. Copeptin parameters in COVID-19 patients

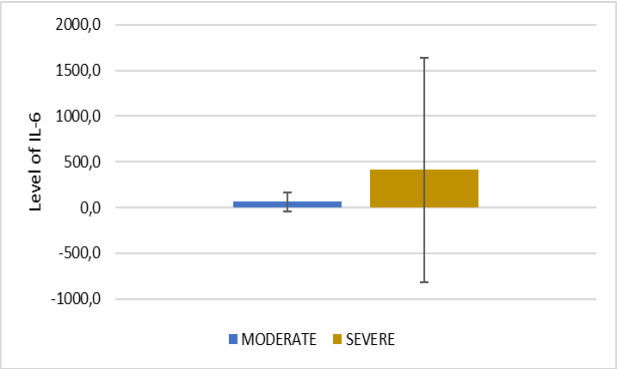

Figure 2. IL-6 parameters in COVID-19 patients

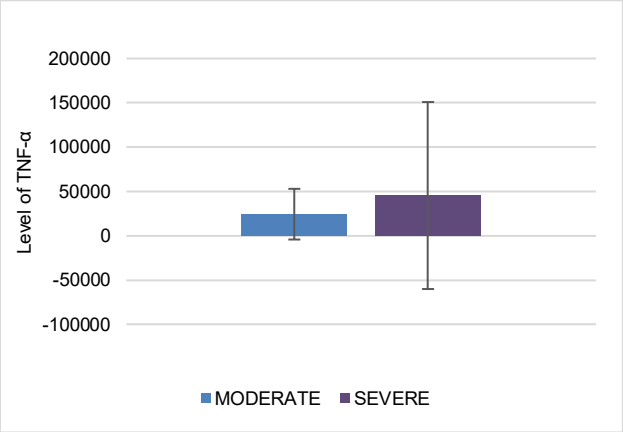

Figure 3. IL-6 parameters in COVID-19 patients

Correlation analysis demonstrated a significant moderate positive correlation between copeptin levels and outcomes (r=0.597; *P*<0.001), a weak and non-significant correlation between IL-6 and outcomes (r=0.239; *P*=0.055), and a negligible and non-significant correlation between TNF-α and outcomes (r=0.140; *P*=0.177).

Table 2. Bonferroni analysis between Copeptin, IL-6, and TNF-α in the outcome of COVID-19 patients

	Copeptin (pmol/L)	IL6 (pg/ml)	TNF- α (pg/mL)
Outcome			
Bonferroni Correlation	0.597	0.239	0.140
P	0.0001*	0.055	0.177
Copeptin (pmol/L)			
Bonferroni Correlation	---	0.044	-0.039
P	---	0.385	0.398
IL6 (pg/ml)			
Bonferroni Correlation	---	---	0.925
P	---	---	0.0001*
TNF-α (pg/ml)			
Bonferroni Correlation	---	0.925	---
P	---	0.0001*	---

Note: *significant with *P*<0.05

DISCUSSION

This study evaluated the correlations of IL-6, TNF-α, and copeptin with clinical outcomes. Multivariate regression was not conducted due to limited samples and data constraints. Disease severity and outcomes were assessed based on cytokine levels, with IL-6 as a key inflammatory mediator. COVID-19 patients showed lower IL-6 levels than those with sepsis, ARDS, or CAR-T CRS. IL-6 signaling involves sgp130 (anti-inflammatory) and trans-signaling (proinflammatory) pathways. Elevated IL-6 and TNF indicate ongoing immune activation and persistent inflammation.[9–11]

Lowering TNF levels influences IL-6, IL-1, adhesion molecules, and VEGF, which are elevated in COVID-19 patients.[12] IL-6, a key cytokine in macrophage activation syndrome (MAS), is increased in both mild and severe cases, while TNF inhibitors have shown therapeutic potential. VEGF and adhesion molecules also contribute to pulmonary capillary leakage. Several hormones, including AVP and cortisol, mediate stress via the hypothalamic-pituitary axis; copeptin, a stable AVP precursor, is easily measurable.[9]

Correlation analysis showed a significant relationship between copeptin and outcomes (r=0.597; *P*<0.001), while IL-6 (r=0.239; *P*=0.055) and TNF-α (r=0.140; *P*=0.177) showed no statistically significant correlation. These findings align with Hammad et al, identifying a 20 pmol/L copeptin cut-off (88.2% sensitivity, 64.9% specificity), which reinforces its value as a COVID-19 severity biomarker.[10] Elevated non-osmotic AVP release may also explain antidiuretic hormone dysregulation in COVID-19 pneumonia.[11,13,14]

Multiple studies report elevated absolute neutrophil counts (ANC) and reduced circulating T cells in severe COVID-19.[15,16] Our findings align and show higher ANC associated with severe cases, likely influenced by IL-6 and TNF-α. Elevated IL-6 activates the hypothalamic-pituitary axis and increases AVP/copeptin and CRH release. Copeptin levels are known to rise in acute infections, especially pneumonia, and are higher in patients with positive blood cultures.[17–21] In this study, copeptin correlated positively but not significantly with IL-6.

Besides that, this study found a very low, non-significant correlation between TNF-α and patient outcomes (r=0.140; *P*=0.177), which is different from Al-Salam et al, who identified TNF-α as a significant link to COVID-19 severity.[22] TNF-α, as a key mediator of inflammation and immune regulation, also drives cytokine synthesis. Dysregulated TNF-α signaling may contribute to cytokine release syndrome (CRS). TNF activates tissue factors in monocytes and endothelial cells, promoting clotting and increasing plasminogen activator inhibitor levels, which suppress fibrinolysis. Hence, the frequent need for anticoagulants in COVID-19.[5,23]

In a study of 54 patients, monocytes continuously produced TNF-α and IL-6, so those with SARS-CoV-2 pneumonia and SRF showed excessive cytokine production and lymphocyte dysregulation, including reduced CD4+ T and B cells. Elevated TNF-α can worsen viral infections, while anti-TNF therapy in mice show reduces inflammation and disease severity. TNF-α inhibitors also modulate effector and regulatory T cell balance in autoimmune diseases such as psoriasis, RA, and IBD.[7]

TNF regulates inflammatory proteins beyond C-reactive protein and serum amyloid A. TNF-targeting aptamers have been shown to improve LPS-induced acute lung injury by reducing neutrophil infiltration, alveolar leakage, and cytokine expression while increasing oxygen saturation. Excessive inflammation, such as cytokine storms, causes severe tissue damage in COVID-19. Combined TNF-α and IFN-γ activation induces strong cell death via the JAK/STAT1/IRF1 pathway, with death rates correlating to cytokine levels. Blocking TNF-α–mediated pathways may help limit inflammatory cell death and tissue injury.[24]

This study supports previous findings that higher copeptin levels correlate with COVID-19 severity and poor prognosis.[25] Copeptin showed a stronger correlation with patient outcomes than IL-6 or TNF-α. Elevated copeptin was linked to worse prognosis, while IL-6 showed only a low, near-significant correlation (r=0.239; *P*=0.055), differing

from Mehta et al., who found IL-6 strongly associated with disease severity.[26] This study suggests that IL-6 is a less sensitive biomarker for COVID-19 outcomes compared to copeptin, with low correlation strength.

Copeptin emerged as the strongest biomarker in this study due to its in vitro stability, long half-life from glycosylation, and multiple epitopes enabling reliable immunoassay detection, making it an ideal surrogate for vasopressin release.[23] Its presence indicates an adverse inflammatory response that activates the hypothalamic-pituitary axis to secrete AVP.[6] In contrast, TNF-α and IL-6 are proinflammatory mediators regulating cell death and immune cell recruitment.[27]

Copeptin reflects stress responses and correlates with cytokines like IL-6 and TNF-α. It has been used to monitor critically ill patients and predict outcomes in pneumonia and COPD, outperforming traditional indicators.[12,25] Adverse COVID-19 outcomes occur only with excessive or prolonged TNF-α and IL-6 signaling. Thus, their elevation alone doesn't always indicate severe infection. Serum copeptin can serve as a practical, reliable biomarker for predicting poor outcomes in moderate to severe COVID-19, aiding prognosis and mortality reduction.[28]

LIMITATION

This study has several limitations, including its retrospective design and small sample size (n=41), which restrict the generalizability of the findings. Additionally, as a single-center study conducted solely at Dr. Moewardi General Hospital, the external validity of the results may be limited. Unaccounted confounding variables may have also influenced the observed correlations. Future research should prioritize large-scale, prospective, multi-center studies involving diverse populations to validate these findings and further elucidate the prognostic value of copeptin in COVID-19.

CONCLUSION

Copeptin emerged as a superior predictive biomarker for clinical outcomes in moderate to severe COVID-19 patients compared to IL-6 and TNF-α. Elevated copeptin levels were significantly associated with poor prognosis. In contrast, IL-6 exhibited only a weak, non-significant correlation with patient outcomes, while TNF-α demonstrated no significant association with disease severity. These findings highlight copeptin's potential as a robust prognostic marker, supporting its clinical utility in the management and risk stratification of COVID-19 patients.

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