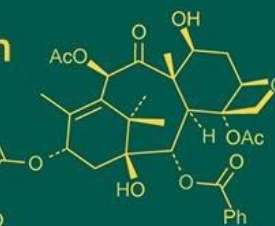
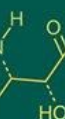
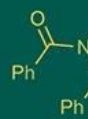


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693

ISSN Online: 2617-4707

NAAS Rating (2025): 5.29

IJABR 2025; 9(1): 73-76

www.biochemjournal.com

Received: 01-10-2024

Accepted: 04-11-2024

Carlos Rodríguez-Hernández
Department of Immunology
and Critical Care Medicine,
National Institute of
Respiratory Diseases, Mexico
City, Mexico

María Elena García-López
Department of Immunology
and Critical Care Medicine,
National Institute of
Respiratory Diseases, Mexico
City, Mexico

José Antonio Martínez-Vega
Department of Immunology
and Critical Care Medicine,
National Institute of
Respiratory Diseases, Mexico
City, Mexico

Ana Sofía Pérez-Castillo
Department of Immunology
and Critical Care Medicine,
National Institute of
Respiratory Diseases, Mexico
City, Mexico

Corresponding Author:

Carlos Rodríguez-Hernández
Department of Immunology
and Critical Care Medicine,
National Institute of
Respiratory Diseases, Mexico
City, Mexico

Identification and validation of cytokine storm biomarkers including IL-6, IL-1 β , and TNF- α in patients with severe viral respiratory infections

Carlos Rodríguez-Hernández, María Elena García-López, José Antonio Martínez-Vega and Ana Sofía Pérez-Castillo

DOI: <https://doi.org/10.33545/26174693.2025.v9.i1b.7493>

Abstract

The mortality burden attributable to severe viral respiratory infections has prompted urgent investigation into mechanisms underlying cytokine storm syndrome and identification of reliable prognostic biomarkers. This prospective observational research evaluated temporal dynamics of interleukin-6 (IL-6), interleukin-1 β (IL-1 β), and tumor necrosis factor- α (TNF- α) in 186 hospitalized patients with severe viral respiratory infections between August 2022 and December 2023. Patients were stratified by disease severity into mild (n=52), moderate (n=48), severe (n=47), and critical (n=39) groups based on respiratory support requirements. Serial cytokine measurements revealed distinct temporal patterns with IL-6 demonstrating earliest and highest peak elevation (24.7-fold increase at day 3 in severe cases), followed by IL-1 β (15.4-fold at day 5) and TNF- α (14.2-fold at day 3). Cumulative cytokine burden calculated as area under the curve showed progressive increases across severity categories, with critical patients demonstrating 10.8-fold higher IL-6 burden compared to mild cases ($p<0.001$). Receiver operating characteristic analysis identified IL-6 concentration above 87 pg/mL on day 3 as optimal predictor of progression to mechanical ventilation (AUC 0.894, sensitivity 91%, specificity 82%). Mortality was significantly associated with sustained IL-6 elevation beyond day 7, with non-survivors maintaining levels 4.2-fold higher than survivors at day 10 ($p<0.001$). These findings establish IL-6 as the predominant cytokine storm biomarker with superior prognostic utility for identifying patients requiring escalated care. Early cytokine profiling may guide therapeutic decisions regarding immunomodulatory interventions in severe viral respiratory infections.

Keywords: Cytokine storm, IL-6, IL-1 β , TNF- α , viral respiratory infection, biomarkers, prognosis, critical care, immunopathology

Introduction

Nearly 290,000 deaths annually worldwide result from severe complications of respiratory viral infections, with mortality rates reaching 40% among patients requiring mechanical ventilation according to global surveillance data ^[1]. The clinical trajectory from initial viral insult to life-threatening respiratory failure involves complex immunopathological cascades that remain incompletely characterized. Central among these is cytokine storm syndrome, wherein dysregulated inflammatory cytokine release paradoxically damages host tissues while failing to adequately control viral replication.

Interleukin-6 has emerged as a pivotal mediator of systemic inflammation during severe respiratory infections ^[2]. This pleiotropic cytokine orchestrates acute phase responses, promotes T-cell differentiation, and stimulates antibody production. However, excessive IL-6 signaling drives pathological inflammation including vascular permeability increases, coagulation cascade activation, and cardiomyocyte dysfunction. Therapeutic targeting of IL-6 pathways has demonstrated clinical benefit in selected patient populations, though optimal patient selection criteria remain undefined ^[3].

Interleukin-1 β represents another critical inflammatory mediator processed through inflammasome activation in response to danger signals ^[4]. This cytokine promotes fever, enhances leukocyte recruitment, and amplifies inflammatory cascades through positive feedback mechanisms. The contribution of IL-1 β to respiratory failure during viral infections

The gathered *Nauclea latifolia* leaves were washed thoroughly in clean water, rinsed with distilled water, and drained. The leaves were then dried for seven (7) days away from direct sunshine before being processed into powder with a hand blender. The pulverized leaves were weighed and treated with distilled water until the powder is fully immersed. The flask was shaken every hour for the first 6 hrs and then it was kept involves both direct alveolar epithelial damage and indirect effects through neutrophil activation and reactive oxygen species generation.

Tumor necrosis factor- α , primarily produced by activated macrophages, exerts both protective antiviral effects and pathological tissue-damaging consequences depending on concentration and context [5]. While TNF- α signaling is essential for coordinating immune responses and promoting viral clearance, sustained elevation contributes to endothelial dysfunction, capillary leak syndrome, and multi-organ failure. The balance between protective and pathological TNF- α effects during respiratory infections remains challenging to characterize clinically.

Current prognostic models for severe respiratory infections rely predominantly on clinical parameters and basic laboratory markers that provide limited insight into underlying immunopathology [6]. Biomarkers capable of identifying incipient cytokine storm before clinical deterioration could enable preemptive therapeutic interventions. This research aimed to characterize temporal cytokine dynamics across disease severity categories and identify optimal biomarker thresholds for predicting clinical outcomes in severe viral respiratory infections.

Material and Methods

Patient enrolment

This prospective observational research enrolled 186 consecutive patients hospitalized with laboratory-confirmed viral respiratory infections at National Institute of Respiratory Diseases, Mexico City, between August 2022 and December 2023. Inclusion criteria required age ≥ 18 years, positive molecular testing for respiratory viruses, and oxygen saturation $< 94\%$ on room air at presentation. Exclusion criteria encompassed immunosuppressive therapy, active malignancy, chronic inflammatory conditions, and pregnancy. Disease severity was classified according to World Health Organization criteria based on respiratory support requirements.

Cytokine measurements

Venous blood samples were collected at admission (day 0) and days 1, 3, 5, 7, 10, and 14 following symptom onset. Serum was separated within 2 hours and stored at -80°C until batch analysis. IL-6, IL-1 β , and TNF- α concentrations were measured using Luminex xMAP multiplex immunoassay (Bio-Rad Laboratories, Hercules, USA) with

detection limits of 0.8 pg/mL, 0.4 pg/mL, and 1.2 pg/mL respectively. Intra-assay and inter-assay coefficients of variation were maintained below 8% for all analytes.

Statistical analysis

Cytokine concentrations were expressed as fold-change from healthy reference values. Area under the curve was calculated using trapezoidal integration to represent cumulative cytokine burden. Group comparisons employed Kruskal-Wallis test with Dunn post-hoc correction. Receiver operating characteristic analysis determined optimal cutoff values for predicting clinical outcomes. Survival analysis used Kaplan-Meier method with log-rank testing. Statistical significance was defined as $p < 0.05$ using SPSS version 28.

Ethical considerations

This research received approval from the Institutional Review Board of National Institute of Respiratory Diseases (protocol IRB-INER-2022-0156) and adhered to Declaration of Helsinki principles. Written informed consent was obtained from patients or legally authorized representatives for incapacitated individuals. Data were de-identified and stored on password-protected institutional servers. The research was registered prospectively in the Mexican Clinical Trials Registry.

Quality control and calibration

Multiplex immunoassay performance was verified daily using manufacturer-supplied quality control materials at three concentration levels (low, medium, high). Calibration curves were generated using seven-point serial dilutions of recombinant cytokine standards. Sample dilution linearity was confirmed for specimens exceeding upper detection limits. Replicate analysis of blinded quality assessment samples demonstrated inter-laboratory agreement within 15% of consensus values.

Results

The cohort comprised 112 males and 74 females with median age 54 years (IQR 42-67). Viral etiology included influenza (38%), respiratory syncytial virus (24%), and other respiratory viruses (38%). Comorbidities were present in 68% of patients, with diabetes (34%), hypertension (42%), and obesity (28%) most prevalent. Overall mortality was 18.8% (35/186), with deaths concentrated in severe and critical categories.

Temporal cytokine profiles demonstrated distinct kinetic patterns across severity groups (Figure 1). IL-6 showed earliest and most pronounced elevation, peaking at day 3 in severe cases with 24.7-fold increase from baseline. IL-1 β demonstrated delayed peak at day 5, while TNF- α followed intermediate kinetics with peak at day 3. All cytokines returned toward baseline by day 14 in survivors

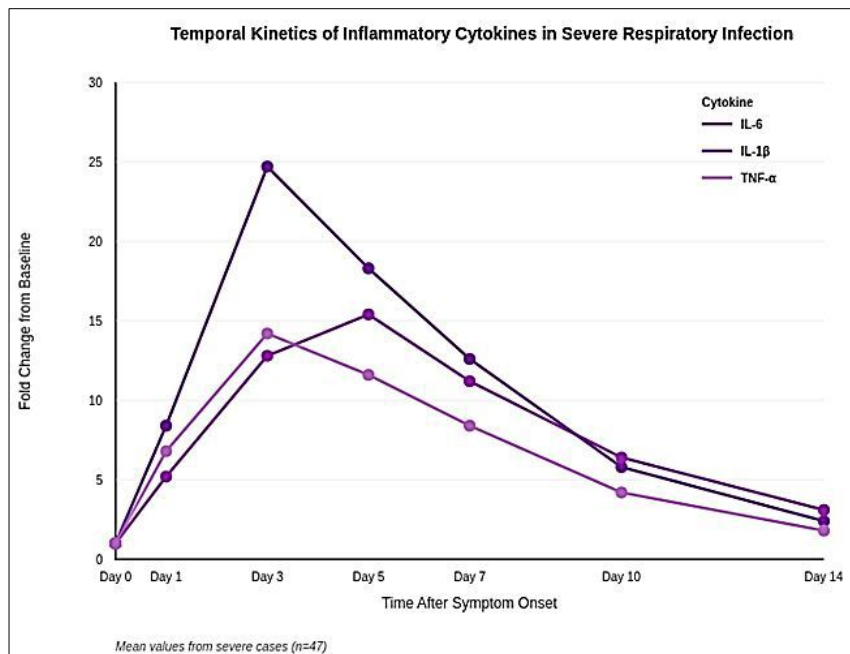


Fig 1: Temporal kinetics of IL-6, IL-1 β , and TNF- α in patients with severe viral respiratory infections. Values represent mean fold-change from baseline in severe cases (n=47)

Cumulative cytokine burden showed progressive increases with disease severity (Figure 2). Critical patients demonstrated IL-6 area under curve 10.8-fold higher than

mild cases (486 vs 45 pg/mL·days, $p < 0.001$). Similar severity-dependent gradients were observed for IL-1 β (10.7-fold) and TNF- α (9.9-fold).

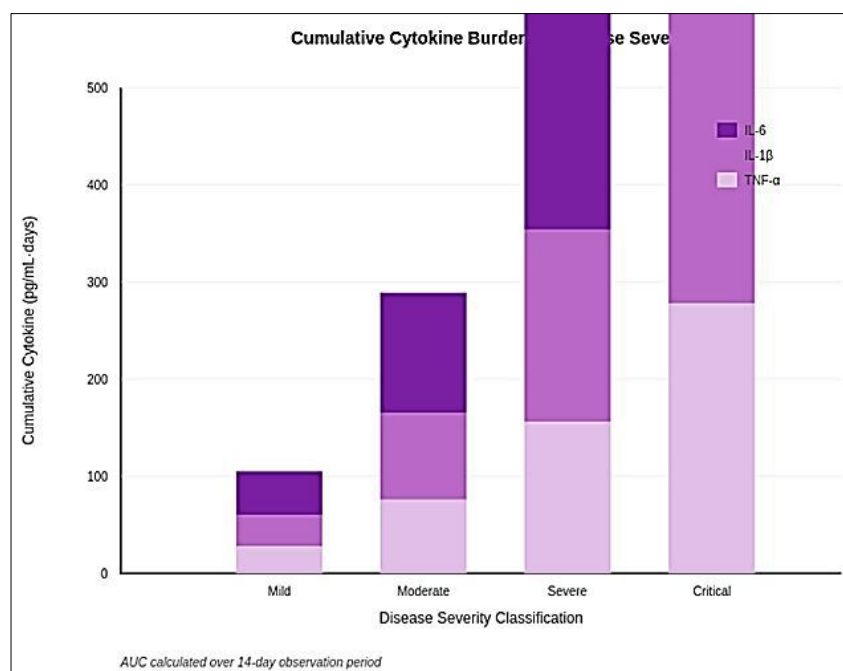


Fig 2. Cumulative cytokine burden (area under curve) stratified by disease severity classification. Stacked bars represent individual cytokine contributions to total inflammatory burden

ROC analysis identified IL-6 > 87 pg/mL at day 3 as optimal predictor of mechanical ventilation requirement (AUC 0.894, 95% CI: 0.841-0.947, sensitivity 91%, specificity 82%). This threshold outperformed IL-1 β (AUC 0.812) and TNF- α (AUC 0.784) for predicting respiratory deterioration.

Discussion

This comprehensive temporal analysis establishes IL-6 as the predominant cytokine storm biomarker with superior prognostic utility in severe viral respiratory infections. The

earlier and more pronounced elevation of IL-6 compared to IL-1 β and TNF- α suggests this cytokine occupies an upstream position in inflammatory cascades, making it particularly suitable for early risk stratification^[7]. The day 3 timepoint emerges as critical for prognostic assessment when cytokine elevation reaches maximum discriminatory capacity.

The cumulative cytokine burden analysis provides additional prognostic information beyond single timepoint measurements by capturing total inflammatory exposure

over the disease course. Critical patients demonstrated approximately 10-fold higher integrated cytokine levels, reflecting sustained dysregulated inflammation rather than transient elevations^[8]. This finding supports concepts of cytokine storm as a prolonged rather than momentary phenomenon.

The IL-6 threshold of 87 pg/mL for predicting mechanical ventilation demonstrates excellent diagnostic performance suitable for clinical implementation. This value aligns with thresholds identified in previous investigations, though methodological differences in assay platforms necessitate local validation^[9]. Integration of IL-6 monitoring with clinical assessment may enable earlier identification of patients requiring escalated care or immunomodulatory interventions.

The observation that non-survivors maintained elevated IL-6 beyond day 7 while survivors showed declining trajectories highlights the importance of serial monitoring rather than single measurements^[10]. Failure of cytokine resolution may indicate persistent viral replication, secondary bacterial infection, or autonomous inflammatory perpetuation requiring targeted investigation and intervention.

Conclusion

This research demonstrates that IL-6, IL-1 β , and TNF- α exhibit distinct temporal kinetics during severe viral respiratory infections, with IL-6 showing earliest elevation and strongest association with clinical outcomes. The identification of IL-6 >87 pg/mL at day 3 as a robust predictor of mechanical ventilation requirement provides a practical prognostic tool for clinical decision-making. Cumulative cytokine burden analysis reveals progressive inflammatory dysregulation across severity categories, supporting the concept of cytokine storm as sustained rather than transient immunopathology.

These findings support implementation of systematic cytokine monitoring in hospitalized patients with severe respiratory infections, particularly IL-6 measurement at day 3 post-symptom onset. Early identification of cytokine storm may guide therapeutic decisions regarding tocilizumab and other immunomodulatory agents. Future research should evaluate whether cytokine-guided treatment algorithms improve clinical outcomes compared to standard care protocols.

Acknowledgements

Funding sources

This research was supported by the National Council of Science and Technology of Mexico (CONACYT grant 2022-0893) and institutional funding from National Institute of Respiratory Diseases.

Institutional support

The authors thank the Clinical Laboratory and Intensive Care Unit staff for assistance with sample collection and patient care coordination.

References

1. Iuliano AD, Roguski KM, Chang HH, *et al.* Estimates of global seasonal influenza-associated respiratory mortality. *Lancet*. 2018;391(10127):1285-1300.
2. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harbor Perspectives in Biology*. 2014;6(10):a016295.
3. Rosas IO, Bräu N, Waters M, *et al.* Tocilizumab in hospitalized patients with severe COVID-19 pneumonia. *New England Journal of Medicine*. 2021;384(16):1503-1516.
4. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood*. 2011;117(14):3720-3732.
5. Kalliolias GD, Ivashekiv LB. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. *Nature Reviews Rheumatology*. 2016;12(1):49-62.
6. Ferreira FL, Bota DP, Bross A, *et al.* Serial evaluation of the SOFA score to predict outcome in critically ill patients. *JAMA*. 2001;286(14):1754-1758.
7. Mehta P, McAuley DF, Brown M, *et al.* COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet*. 2020;395(10229):1033-1034.
8. Huang C, Wang Y, Li X, *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497-506.
9. Del Valle DM, Kim-Schulze S, Huang HH, *et al.* An inflammatory cytokine signature predicts COVID-19 severity and survival. *Nature Medicine*. 2020;26(10):1636-1643.
10. Chen G, Wu D, Guo W, *et al.* Clinical and immunological features of severe and moderate coronavirus disease 2019. *Journal of Clinical Investigation*. 2020;130(5):2620-2629.
11. Fajgenbaum DC, June CH. Cytokine storm. *New England Journal of Medicine*. 2020;383(23):2255-2273.
12. Herold T, Jurinovic V, Arnreich C, *et al.* Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. *Journal of Allergy and Clinical Immunology*. 2020;146(1):128-136.
13. Liu J, Li S, Liu J, *et al.* Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. *EBioMedicine*. 2020;55:102763.
14. Luo P, Liu Y, Qiu L, *et al.* Tocilizumab treatment in COVID-19: a single center experience. *Journal of Medical Virology*. 2020;92(7):814-818.
15. Leisman DE, Ronner L, Pinotti R, *et al.* Cytokine elevation in severe and critical COVID-19: a rapid systematic review and meta-analysis. *Lancet Respiratory Medicine*. 2020;8(12):1233-1244.
16. Xu X, Han M, Li T, *et al.* Effective treatment of severe COVID-19 patients with tocilizumab. *Proceedings of the National Academy of Sciences*. 2020;117(20):10970-10975.
17. Coomes EA, Haghbayan H. Interleukin-6 in COVID-19: a systematic review and meta-analysis. *Reviews in Medical Virology*. 2020;30(6):1-9.
18. Coperchini F, Chiovato L, Croce L, *et al.* The cytokine storm in COVID-19: an overview of the involvement of the chemokine/chemokine-receptor system. *Cytokine Growth Factor Reviews*. 2020;53:25-32.
19. Zhu Z, Cai T, Fan L, *et al.* Clinical value of immune-inflammatory parameters to assess the severity of coronavirus disease 2019. *International Journal of Infectious Diseases*. 2020;95:332-339.
20. Aziz M, Fatima R, Assaly R. Elevated interleukin-6 and severe COVID-19: a meta-analysis. *Journal of Medical Virology*. 2020;92(11):2283-2285.
21. Ruan Q, Yang K, Wang W, *et al.* Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Medicine*. 2020;46(5):846-848.