Salivary Cytokine Profiles as Early Diagnostic Biomarkers for Sepsis Severity in Emergency Department Patients

Hom Sai Guo
rosehomsai@gmail.com
April 30, 2026

Abstract:
Globally, sepsis is one of the deadliest and most expensive diseases, resulting in around 11 million annual deaths (Rudd et al., 2020). Current blood-based biomarkers are invasive, sensitive to time and often do not provide clear results in the early phase of treatment (Wacker et al., 2013). This study proposes a potential clinical trial aiming to test the hypothesis that salivary levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interleukin-10 (IL-10) at emergency department (ED) admission are associated with Sequential Organ Failure Assessment (SOFA) scores and predict early 28-day death in adults with sepsis. This study will use saliva as a non-invasive, rapidly accessible biofluid and multiplex cytokine immunoassay technology to provide a proof-of-concept of point-of-care salivary diagnostics in sepsis triage. This method could alter early sepsis practice, especially in resource-limited settings.

Introduction:

Despite advances, sepsis continues to be a leading cause of death. Recent biomarkers, including procalcitonin and serum lactate, are invasive and have serious shortcomings in early sensitivity and specificity (Pierrakos & Vincent, 2010). Sepsis pathophysiology occurs under the influence of an irregular cytokine storm comprising IL-6, TNF-α, and IL-10 (Chousterman et al., 2017), but this has not yet been explored in saliva, a non-invasive biofluid that reflects systemic inflammation (Song et al., 2023). Although salivary cytokines are useful in chronic inflammatory diseases (Dongiovanni et al., 2023), their use in acute sepsis remains understudied. Therefore, this study asks: Do salivary concentrations of IL-6, TNF-α, and IL-10 measured at ED admission correlate with SOFA scores and predict 28-day all-cause mortality in adult patients meeting Sepsis-3 diagnostic criteria?

Literature Review:

Sepsis pathophysiology involves pro-inflammatory and immunosuppressive cytokine signaling. Schulte et al. (2013) show that IL-6, TNF-alpha, and IL-10 are important regulators of immune cascade during sepsis. High concentrations of the three indicate organ dysfunction and mortality. Tanaka et al. (2014) also found IL-6 as a central intermediary of the acute-phase response and a biomarker action target. Such cytokines can be found not only in blood but also in mucosal secretions due to systemic immune responses and local glandular responses (Yoshizawa et al., 2013).

The diagnostic capability of saliva has received considerable momentum over the last few years. Song et al. (2023) compiled a list of diverse systemic diseases for which salivary biomarkers are promising participants in the diagnostic process. Likewise, Dongiovanni et al. (2023) summarized data regarding salivary cytokines as non-invasive indicators of chronic low-grade inflammation and concluded that salivary IL-6 and TNF-α represent an inflammatory burden in the system with a moderate-good diagnostic measure. In an acute care environment, Tzira et al. (2018) discovered that physiological stress reactions are associated with salivary biomarkers in critically ill children, which provides initial evidence that saliva could measure changes in the acute system.

Methodologically, multiplex immunoassays using Luminex bead-based systems allow high-sensitivity detection of multiple cytokines in small samples (Leng et al., 2008; Günther et al., 2020). This technology is highly appropriate for salivary matrices and for point-of-care adaptation. Recent point-of-care diagnostics are, however, based on blood, and few efforts have been made to explore salivary testing platforms, even though the latter have ideal logistical features compared to blood-based tests (Moore et al., 2025; Alter, 2021). SOFA score, the most tested severity scale in sepsis (Seymour et al., 2016; Vincent et al., 1996), is the endpoint of choice in studies of biomarker correlation under the Sepsis-3 consensus definitions (Singer et al., 2016). Povoa et al. (2023) noted that future research on sepsis biomarkers needs to be framed in clinical terms, with SOFA and 28-day mortality as the

appropriate metrics. Taken together, the literature provides a rationale for studying the salivary cytokine panel to assess the severity of sepsis and confirms a lack of prospective clinical studies in the area.

Methodology:

The present research will use a prospective observational cohort with a minimum duration of 18 months in two tertiary urban hospital EDs. Patients meeting Sepsis-3 criteria (adults older or equal to 18 years with suspected sepsis and an acute SOFA increase of more than or equal to 2 points) are eligible (Singer et al., 2016). Exclusion criteria include: oral surgery or oral infection, autoimmune disease, immunosuppressive treatment, or inability to consent.

Unstimulated whole saliva will be obtained via the passive drool method. It will be collected within 60 minutes of ED triage, before the administration of antibiotics. The samples will then be immediately centrifuged, aliquoted, and stored at −80 °C until batch analysis. At the same time, conventional clinical information will be gathered, including vital signs, laboratory values, and the components of the SOFA score at 0, 24, and 72 hours. Comparators will include blood cultures, serum procalcitonin, serum IL-6, lactate, and CRP.

A Luminex-based multiplex bead immunoassay panel for IL-6, TNF-α, and IL-10 will be used (Leng et al., 2008; Günther et al., 2020) because it can simultaneously analyze several analytes in micro-volume samples with a femtogram-level sensitivity limit. The statistical tests to be conducted are Spearman rank correlation between salivary levels of cytokines and SOFA scores, receiver operating curve analysis with the determination of the area under the curve (AUC) to predict 28-day mortality, and multivariate logistic regression, which will control the effects of age, sex, and infection source. The preliminary results of the cytokine correlation evidence of similar studies provide an approximation of 200 as a target number of patients, estimated to reach 80% power at $\alpha = 0.05$ (Slavish et al., 2015; Tzira et al., 2018). This research will be submitted to the Institutional Review Board (IRB), and all participants will sign the informed consent form.

Expected Results:

The hypothesis is based on the fact that salivary IL-6 will show the most statistically significant and strongest correlation with SOFA score at admission. Specifically, the Spearman ρ is expected to be 0.55 or greater due to the proven role of IL-6 as the leading acute-phase cytokine in sepsis (Tanaka et al., 2014; Schulte et al., 2013). Salivary TNF-α should correlate with early organ dysfunction, and salivary IL-10, as an anti-inflammatory mediator, is likely to demonstrate the likelihood of an anti-inflammatory shift and immunosuppressive immune paralysis in non-survivors. ROC analysis should yield AUC values of 0.75 or above for salivary IL-6, which would be similar to the performance thresholds that serum procalcitonin can achieve in 28-day mortality prediction as reported by Wacker et al. (2013). Subgroup analyses will consider that the release of salivary cytokines may differ depending on the source of infection (e.g., pulmonary vs. abdominal sepsis) and that combinations of cytokine signatures may be more effective than single markers. These numerical data will be reported with 95 % confidence intervals, and forest plots will be used to visualize subgroup effects.

Conclusion:

This study proposes a non-invasive salivary cytokine panel to address the critical gap in early sepsis stratification. If successful, this approach could eliminate needle-based collection and enable rapid point-of-care testing (Moore et al., 2025; Alter, 2021). Beyond clinical use, this study would establish a methodological archetype for the urgent field of salivary diagnostics in acute infectious disease (Póvoa et al., 2023). Saliva-based testing is low in cold-chain requirements and does not need phlebotomy infrastructure, allowing applications in low- and middle-income countries where sepsis burden is highest (Rudd et al., 2020).

References

Alter, D. N. (2021). Point-of-care testing for the emergency department patient: Quantity and quality of the available evidence. Archives of Pathology & Laboratory Medicine, 145(3), 308–319. https://doi.org/10.5858/arpa.2020-0495-RA

Chousterman, B. G., Swirski, F. K., & Weber, G. F. (2017). Cytokine storm and sepsis disease pathogenesis. Seminars in Immunopathology, 39(5), 517–528. https://doi.org/10.1007/s00281-017-0639-8

Dongiovanni, P., Meroni, M., Casati, S., Goldoni, R., Thomaz, D. V., Kehr, N. S., Galimberti, D., Del Fabbro, M., & Tartaglia, G. M. (2023). Salivary biomarkers: Novel noninvasive tools to diagnose chronic inflammation. International Journal of Oral Science, 15, Article 27. https://doi.org/10.1038/s41368-023-00231-6

Fleischmann, C., Scherag, A., Adhikari, N. K. J., Hartog, C. S., Tsaganos, T., Schlattmann, P., Angus, D. C., & Reinhart, K. (2016). Assessment of global incidence and mortality of hospital-treated sepsis: Current estimates and limitations. American Journal of Respiratory and Critical Care Medicine, 193(3), 259–272. https://doi.org/10.1164/rccm.201504-0781OC

Günther, A., Becker, M., Göpfert, J., Joos, T., & Schneiderhan-Marra, N. (2020). Comparison of bead-based fluorescence versus planar electrochemiluminescence multiplex immunoassays for measuring cytokines in human plasma. Frontiers in Immunology, 11, Article 572634. https://doi.org/10.3389/fimmu.2020.572634

Leng, S. X., McElhaney, J. E., Walston, J. D., Xie, D., Fedarko, N. S., & Kuchel, G. A. (2008). ELISA and multiplex technologies for cytokine measurement in inflammation and aging research. The Journals of Gerontology: Series A, 63(8), 879–884. https://doi.org/10.1093/gerona/63.8.879

Moore, T. H. M., Dawson, S., Kirby, K., Body, R., Thompson, A., Adepoju, Y. O., Perry, R., Nicholson, H., Dinnes, J., Mitchell, K., Savičović, J., Voss, S., & Benger, J. R. (2025). Point-of-care tests in the emergency medical services: A scoping review. Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine, 33, Article 18. https://doi.org/10.1186/s13049-025-01329-y

Pierrakos, C., & Vincent, J.-L. (2010). Sepsis biomarkers: A review. Critical Care, 14(1), Article R15. https://doi.org/10.1186/cc8872

Póvoa, P., Coelho, L., Dal-Pizzol, F., Ferrer, R., Huttner, A., Conway Morris, A., Nobre, V., Ramirez, P., Rouze, A., Salluh, J., Singer, M., Sweeney, D. A., Torres, A., Waterer, G., & Kalil, A. C. (2023). How to use biomarkers of infection or sepsis at the bedside: Guide to clinicians. Intensive Care Medicine, 49(2), 142–153. https://doi.org/10.1007/s00134-022-06956-y

Rudd, K. E., Johnson, S. C., Agesa, K. M., Shackelford, K. A., Tsoi, D., Kievlan, D. R., Colombara, D. V., Ikuta, K. S., Kissoon, N., Finfer, S., Fleischmann-Struzek, C., Machado, F. R., Reinhart, K., Rowan, K., Seymour, C. W., Watson, R. S., West, T. E., Marinho, F., Hay, S. I., … Naghavi, M. (2020). Global, regional, and national sepsis incidence and mortality, 1990–2017: Analysis for the Global Burden of Disease Study. The Lancet, 395(10219), 200–211. https://doi.org/10.1016/S0140-6736(19)32989-7

Schulte, W., Bernhagen, J., & Bucala, R. (2013). Cytokines in sepsis: Potent immunoregulators and potential therapeutic targets—An updated view. Mediators of Inflammation, 2013, Article 165974. https://doi.org/10.1155/2013/165974

Seymour, C. W., Liu, V. X., Iwashyna, T. J., Brunkhorst, F. M., Rea, T. D., Scherag, A., Rubenfeld, G., Kahn, J. M., Shankar-Hari, M., Singer, M., Deutschman, C. S., Escobar, G. J., & Angus, D. C. (2016). Assessment of clinical criteria for sepsis: For

the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA, 315(8), 762–774. https://doi.org/10.1001/jama.2016.0288

Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., Bauer, M., Bellomo, R., Bernard, G. R., Chiche, J.-D., Coopersmith, C. M., Hotchkiss, R. S., Levy, M. M., Marshall, J. C., Martin, G. S., Opal, S. M., Rubenfeld, G. D., van der Poll, T., Vincent, J.-L., & Angus, D. C. (2016). The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA, 315(8), 801–810. https://doi.org/10.1001/jama.2016.0287

Slavish, D. C., Graham-Engeland, J. E., Smyth, J. M., & Engeland, C. G. (2015). Salivary markers of inflammation in response to acute stress. Brain, Behavior, and Immunity, 44, 253–269. https://doi.org/10.1016/j.bbi.2014.08.008

Song, M., Bai, H., Zhang, P., Zhou, X., & Ying, B. (2023). Promising applications of human-derived saliva biomarker testing in clinical diagnostics. International Journal of Oral Science, 15, Article 2. https://doi.org/10.1038/s41368-022-00209-w

Tanaka, T., Narazaki, M., & Kishimoto, T. (2014). IL-6 in inflammation, immunity, and disease. Cold Spring Harbor Perspectives in Biology, 6(10), Article a016295. https://doi.org/10.1101/cshperspect.a016295

Tzira, D., Prezerakou, A., Papadatos, I., Vintila, A., Bartzeliotou, A., Apostolakou, F., Papassotiriou, I., & Papaevangelou, V. (2018). Salivary biomarkers may measure stress responses in critically ill children. SAGE Open Medicine, 6, 2050312118802452. https://doi.org/10.1177/2050312118802452

Vincent, J.-L., Moreno, R., Takala, J., Willatts, S., De Mendonça, A., Bruining, H., Reinhart, C. K., Suter, P. M., & Thijs, L. G. (1996). The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. Intensive Care Medicine, 22(7), 707–710. https://doi.org/10.1007/BF01709751

Wacker, C., Prkno, A., Brunkhorst, F. M., & Schlattmann, P. (2013). Procalcitonin as a diagnostic marker for sepsis: A systematic review and meta-analysis. The Lancet Infectious Diseases, 13(5), 426–435. https://doi.org/10.1016/S1473-3099(12)70323-7

Yoshizawa, J. M., Schafer, C. A., Schafer, J. J., Farrell, J. J., Paster, B. J., & Wong, D. T. W. (2013). Salivary biomarkers: Toward future clinical and diagnostic utilities. Clinical Microbiology Reviews, 26(4), 781–791. https://doi.org/10.1128/CMR.00021-13