

Characterizing the kinetics of presepsin and associated inflammatory biomarkers in human endotoxemia

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Abstract

In this study, we describe the kinetic new potential inflammatory biomarker, presepsin, together with a panel of well-established biomarkers in a human endotoxemia study. We evaluated and identified biomarker correlations that could hold valuable insight regarding state of inflammation and infection when considered in combination. Our study builds towards using biomarkers as treatment response biomarkers.

Introduction

Severe bacterial infections and sepsis are characterized by a systemic immune response. Inflammation-associated proteins can hold potential as treatment efficacy biomarkers. Such biomarkers can be utilized to monitor antibiotic therapy and inform treatment optimization, aiming to improve outcomes in patients¹. It is essential that treatment efficacy biomarkers have a short induction time, a relatively rapid half-life, and closely follow the course of infection to reflect treatment response and/or disease progression.

The currently used biomarkers of infection, such as leukocyte counts and C-reactive protein (CRP), are either non-specific or suffer from a delayed onset of production and slow half-life. Identifying novel biomarkers with more favourable properties could improve the utility of biomarker guided treatments in severe infections and sepsis. Additionally, using a combination of different biomarkers has been shown to improve predictive performance², suggesting the importance of understanding correlation in biomarker kinetics. Presepsin is emerging as a potential biomarker to inform treatment of infections and sepsis, and is associate with clinical disease severity^{2,3}. However, the kinetics of presepsin, and how it relates to other established biomarkers, is poorly understood.

The clinical utility of treatment response biomarkers for infection and sepsis are currently hampered by poor characterization of their kinetics. Characterizing biomarker kinetics in severely ill and septic patient is challenging due to the heterogeneity in underlying infection or disease state. Experimental endotoxemia in healthy volunteers resembles some features of the inflammatory response during infection and sepsis, and may help to better understand specific components of the inflammatory responses that play a critical role in sepsis. In this model, a systemic toll-like receptor 4 (TLR-4) mediated inflammatory response is induced by administering lipopolysaccharide (LPS).

In the current study, we evaluated presepsin response in a human LPS challenge model, related the response to more standard inflammatory biomarkers, and characterized the kinetics of the response.

Materials And Methods

This is a sub-study of a larger previously published study⁴. This sub-study included ten healthy male volunteers who received a single dose of 1 or 2 ng/kg bodyweight of LPS. Blood samples were collected

pre-LPS exposure up to 24 hours post LPS-administration. Further study details can be found in the primary publication⁴.

We quantified plasma concentration-time profiles of presepsin by ELISA (Abbexa, abx76557, limit of quantification (LOQ) = 31.25 pg/mL). Inflammatory cytokines (IL-1 β , IL-1Ra, IL-6, IL-8, and TNF- α) and CRP were analysed using electrochemiluminescence assays, as previously described⁴.

We calculated the total area under concentration-time curve (AUC) post-LPS administration for each individual volunteer and biomarker, using the trapezoidal method with observations below the LOQ imputed by LOQ/2. We compared AUC between the dose groups (Wilcoxon Rank Sum Test, $\alpha = 0.05$), and calculated the Pearson correlation (r) between the different biomarkers and applied hierarchical clustering.

Results

An increase for all measured inflammatory biomarkers was observed (Fig. 1A), with an observed maximum response within 10 hours post-LPS administration except for CRP. For IL-1ra, its peak exceeded the upper LOQ of 2250 pg/mL and was therefore excluded from the AUC calculations.

We identified significant effect of LPS dose and mean biomarker AUC for TNF- α , IL-10, and IL-8 (Fig. 1B), where the relative difference in AUC between the dose groups was 290%, 285%, and 252%, respectively. A positive correlation between individual-level AUC was observed for all biomarkers (Fig. 1C). The AUC of TNF- α was highly and significantly ($p < 0.05$) correlated to both IL-1 β , IL-8, and IL-10 ($r \geq 0.85$). These findings align with previous *in vitro* studies where IL-1 β and TNF- α induce each other's expression^{5,6} and TNF- α enhances IL-8 expression, while anti-TNF drugs has been shown to suppress LPS-induced IL-8 secretion⁷. The strong correlation between IL-1 β and IL-8 ($r = 0.94$) could be a result of TNF- α -related induction, but could also be affected by direct interaction between the two inflammatory markers. Presepsin was most strongly correlated with IL-1 β ($r = 0.73$) and IL-8 ($r = 0.82$), and all three markers clustered in the correlation analysis. Due to the strong correlation, the clinical value of these biomarkers could potentially be increased when used in combination.

Discussion

In this study, we describe the kinetic and LPS dose-response of a new potential inflammatory biomarker, presepsin, together with a panel of well-established biomarkers. To our knowledge, this is the first study that describes human presepsin kinetics in healthy volunteers after LPS administration. The study adds value as simultaneously measuring levels of a large panel of host response markers in the same individuals allows for the evaluation of individual-level biomarker correlations. Such correlations can aid in the understanding of the complex interplay between biomarkers, as demonstrated by the identification of known interactions between TNF- α , IL-1 β , and IL-8.

Our study builds towards an increased understanding of presepsin kinetics in response to LPS and how it relates to established inflammatory biomarkers. Although our study only covers a part of the complex picture of immune response to infection by design, it provides an important piece of the puzzle and endorses presepsin as a treatment response biomarker candidate. The rapid pronounced induction and short half-life of presepsin support further studies of presepsin as a biomarker for systemic inflammation. Previous studies have reported higher presepsin levels in patients with infection compared to non-infected patients³, thus indicating specificity for infections. Presepsin specificity gives it an advantage as a potential treatment response biomarker over more general biomarkers of inflammation, such as CRP.

In conclusion, our findings characterizing the kinetics of inflammatory markers may constitute a step towards understanding parts of the TLR-4 mediated immune response during an infection. Such understanding can contribute towards the efforts of interpretation of clinical inflammatory biomarkers, which in the future could play a role in informing treatment optimization and individualization for patients with severe infections or sepsis.

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Figures

Lipopolysaccharide (LPS)-induced host response in healthy volunteers (n=10). **A.** concentration-time profiles of median (solid line) and individual (shaded lines) concentration of host response markers. **B.** 24-hour post-LPS area under the curve (AUC) per host response marker, with dose group comparison (The Wilcoxon Rank Sum Test), ns: $p > 0.05$, *: $p \leq 0.05$, **: $p \leq 0.01$ **C.** Hierarchically clustered Pearson correlation (Corr) matrix of host response marker AUCs, X: $p > 0.05$