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Ex Vivo Endotoxin Stimulation of Blood for Predicting Survival in Patients With Sepsis:

A Systematic Review

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Abstract

BACKGROUND: Sepsis is a syndrome characterized by host immune dysfunction, with the extent of immunoparalysis differing among patients. Lipopolysaccharide (LPS) is used commonly to assess the immune function of critically ill patients with sepsis. However, the reliability of this ex vivo diagnostic test in predicting clinical outcomes remains uncertain.

RESEARCH QUESTION: Does LPS-induced tumor necrosis factor (TNF) production from the blood of patients with sepsis predict mortality? Secondary outcomes included ICU and hospital stay durations, nosocomial infection rate, and organ recovery rate.

STUDY DESIGN AND METHODS: Human sepsis studies from various databases through April 2023 were evaluated. Inclusion criteria encompassed LPS-stimulated blood assays, English language, and reported clinical outcomes. Bias risk was evaluated using the Newcastle-Ottawa scale (NOS). Relationships between TNF production and mortality were analyzed at sepsis onset and during established sepsis, alongside secondary outcomes.

RESULTS: Of 11,580 studies, 17 studies (14 adult and three pediatric) were selected for analysis. Although 15 studies were evaluated as moderate to high quality using the NOS, it is important to note that some of these studies also had identifiable biases, such as unclear methods of participant recruitment. Nine studies detailed survival outcomes associated with LPS-induced TNF

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Additional information: The e-Appendixes are available online under “Supplementary Data.”

production at sepsis onset, whereas five studies explored TNF production's relationship with mortality during established sepsis. Trends suggested that lower LPS-induced TNF production correlated with higher mortality. However, heterogeneity in methodologies, especially the LPS assay protocol, hindered definitive conclusions. Publication bias was highlighted using funnel plot analysis. Concerning secondary outcomes, diminished TNF production might signify worsening organ dysfunction, although the link between cytokine production and nosocomial infection varied among studies.

INTERPRETATION: For functional immune profiling in sepsis, streamlined research methodologies are essential. This entails organizing cohorts based on microbial sources of sepsis, establishing standardized definitions of immunoparalysis, using consistent types and dosages of immune stimulants, adhering to uniform blood incubation conditions, and adopting consistent clinical outcomes.

Keywords

clinical outcomes; endotoxin; ex vivo stimulation; mortality; sepsis

Sepsis, the leading cause of in-hospital mortality, is responsible for 11 million global deaths annually.[1–3] In the United States alone, the management of sepsis incurs a conservative estimate of $62 billion in health care costs, surpassing all other disease states in terms of financial impact.[1,2,4] Immune dysfunction (persistent inflammation, immunosuppression, or both) during sepsis contributes to most of the disease's morbidity and mortality, because patients become increasingly susceptible to pathogens that usually are eliminated rapidly in healthy patients.[5,6] At the time of death, approximately 80% of patients have evidence of an unresolved septic focus,[7] and secondary infections complicate the clinical course of approximately 13.5% of patients with sepsis and 39% of patients with septic shock.[8,9]

The cause of secondary infection is believed to be a phenomenon called immunoparalysis.[10,11] This represents the impaired ability to mount an appropriate immune response after exposure to damage- and pattern-associated molecular patterns.[12,13] Immunoparalysis may result from the combined effects of T-lymphocyte anergy, tumor necrosis factor (TNF)-initiated release of antiinflammatory IL-10, and apoptosis of monocytes and other immune cells.[10,11,14,15] The tremendous heterogeneity of immune responses in patients with sepsis, combined with the high disease burden imposed by sepsis, has fueled the need to develop blood tests that interrogate patients' immune function with the hope of identifying those who show a compromised immune response. Clinicians then may administer adjuvant therapy to enhance the response in these immune-paralyzed patients, aiming to reduce secondary infections and improve clinical outcomes.[16–19]

Lipopolysaccharide (LPS, or bacterial endotoxin) is a proinflammatory molecule that was described first in 1975.[20] It binds to the toll-like receptor 4 pattern recognition receptor and activates MyD88, thereby triggering intracellular nuclear factor-κB-activated transcription of proinflammatory molecules such as TNF (formerly TNF-α).[21,22] This highly reproducible inflammatory pathway has formed the basis of hundreds of murine research investigations seeking to emulate the mechanism underlying human sepsis. Although the latter practice recently has fallen out of favor,[23,24] LPS continues to be used to gauge the integrity of

critically ill patients' immune function with the aim of identifying patients who could benefit from therapeutic intervention.[25] This immune assessment involves the exposure of blood from patients with sepsis to LPS ex vivo, followed by quantification of the cytokines produced by the blood sample. Impaired cytokine production may signal compromised cellular immunity and an increased risk of adverse clinical outcomes. However, the relatively small sizes of observational research cohorts used to test this hypothesis, as well as the variable definitions of clinically significant outcomes, have left us without conclusive answers.

Therefore, the objective of this investigation was to evaluate whether prior studies support the continued use of ex vivo blood stimulation to predict clinical outcomes in critically ill patients with sepsis. Specifically, we aimed to assess the association between LPS-induced TNF production and mortality (primary outcome), nosocomial infection rate, ICU or hospital length of stay, and persistent organ dysfunction (secondary outcomes).

Study Design and Methods

Eligibility Criteria

The study was designed and conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 guidelines.[26] All human sepsis studies (diagnosis by Sepsis-1, Sepsis-2, or Sepsis-3 criteria) were screened for inclusion.[27–29] Studies were excluded if they comprised a conference abstract alone; if they did not include whole blood or peripheral blood mononuclear cell stimulation by LPS; if cytokine concentrations were not measured after LPS stimulation; if they did not measure or report a clinical outcome, or both; and if they were not written in English. Given that mortality (in-hospital, in-ICU, or 28-day mortality) is an objective and commonly reported clinical outcome, it was selected as the primary outcome variable. Secondary outcomes included nosocomial infections, ICU or hospital length of stay, and long-term organ dysfunction as defined by Sequential Organ Failure Assessment or Acute Physiology and Chronic Health Evaluation II severity of disease classification system.[30,31]

Information Sources and Search Strategy

Searches were performed in MEDLINE, Web of Science, Cochrane, and Embase databases. Studies up to and including April 2023 were included. The systemic review was registered on the International Prospective Register of Systematic Reviews international prospective register and can be accessed publicly online.[32] Key search terms included *sepsis*, *septic shock*, *whole blood stimulation*, and *lipopolysaccharides* (or *endotoxin*). A research librarian (A. K.) conducted searches using these terms and their synonyms (e-Appendix 1).

Selection Process

Covidence software (Veritas Health Innovation) was used to compile studies identified by the search for further evaluation. This software allowed two reviewers (J. W. and A. S. B.) to screen studies, extract data, and assess bias independently. Disagreements between reviewers were resolved by a third, independent reviewer (E. S. H.), in a similarly masked fashion.

Data Collection Process

A data extraction template (e-Appendix 2) was constructed using Covidence software and was used to compile data from each article that satisfied full-text screening criteria. Key data that were extracted included study design, population descriptors, cohort size, concentration of cytokines measured, potential conflicts of interest, and clinical outcomes.

Data Items

Cytokine concentrations, measured in response to ex vivo LPS stimulation, were reported with respect to the day of sepsis diagnosis, up to a maximum of 14 days after diagnosis. The bacterial origin of LPS, its dose, and the stimulation conditions also were noted. All reported clinical outcomes were compiled from the included studies.

Study Risk of Bias Assessment

Risk of bias was scored independently by two investigators (J. W. and A. S. B.) using the Newcastle-Ottawa scale (NOS) for cohort studies (e-Appendix 3).[33,34] This tool was selected because of the nonrandomized design of the prospective cohort studies qualifying for inclusion. The NOS assigns a scores of 1 through 9 based on selection, comparability, and outcomes domains. The score generated by each investigator for each domain was used to generate a mean score for that domain. Each mean domain score then summed to generate a total NOS score. This method was used so that deficiencies or strengths in one domain would not disproportionately influence the overall NOS score. Studies scoring < 4 were considered low quality, those with scores of 4 to 6 were considered moderate quality, and those with score of > 6 were considered high quality.

Effect Measures and Synthesis Methods

Cytokine concentrations and LPS doses and durations were quantified as continuous variables, and mean values were reported. Aggregate mortality data and nosocomial infections were reported as binary outcomes, whereas ICU or hospital length of stay and organ dysfunction scores were continuous. A forest plot was constructed to synthesize data from studies quantifying LPS-induced TNF production. Because of the use of different cytokine quantification methods, the standardized mean difference was used to compare the association between LPS-induced TNF and clinical outcomes.

Several studies conducted ex vivo stimulation tests at different intervals after diagnosing septic critical illness and patient enrollment into the study. Given the variability in sepsis definitions, which potentially leads to differences in the timing of meeting sepsis criteria, we categorized the progression of immune function in sepsis into two stages: sepsis onset (generally characterized as the initial 1–2 days after sepsis diagnosis and patient inclusion) and established sepsis (marked as 6–8 days after sepsis diagnosis and patient enrollment). Statistical analysis was performed using the *meta* package version 6.5–0 for R software (R Foundation for Statistical Computing).[35]

Certainty Assessment

We used funnel plots to detect biases resulting from selective publication, heterogeneity, or small study effects. For example, if smaller studies tend to show larger effect sizes, this could indicate a potential small study effect, which might arise because of bias or random chance.

Results

A total of 12,163 studies were extracted, of which 583 duplicates were removed by Covidence software in an automated fashion, to yield 11,580 unique studies for title and abstract screening (Fig 1). Forty-one of these studies met criteria for full-text review, of which 17 studies were confirmed to meet inclusion and exclusion criteria. The proportionate agreement of interrater reliability (J. W. and A. S. B.) during the title and abstract screening phase was 0.99, and during the full-text review phase, it was 0.68, with conflicts resolved by the third reviewer (E. S. H.).

Association Between LPS-Induced TNF Concentration at Sepsis Onset and Mortality

Of 17 included studies, 14 reported survival outcomes (Table 1). Five of these 14 studies were excluded from forest analysis because they did not measure TNF production,[36–38] did not report variance in measured TNF concentrations,[25] or described survival results in the text but did not show corresponding numerical data.[39] The remaining nine studies yielded a total of 487 clinical observations (Fig 2A). Three of these studies reported ICU mortality,[40–42] four studies reported in-hospital mortality,[43–46] and two studies reported 28-day mortality.[47,48]

Considerable variability existed in both the timing of blood samples obtained from qualified patients and the techniques used for immune cell activation. As closely aligned with the original article's phrasing as possible, the fourth column of Table 1 indicates that the initial blood samples were collected within a range of < 24 h to < 72 h after the onset of sepsis or ICU admission. In several instances, it remained ambiguous whether the timing of blood collection was measured from the diagnosis of sepsis, from ICU admission, or from a concurrent diagnosis of sepsis leading to ICU admission. This level of inconsistency is particularly noteworthy in the rapidly evolving context of diseases like sepsis.

Although eight of the nine studies used whole blood to assess immune responses, five studies used LPS derived from *Salmonella abortusequi*, two used LPS derived from *Escherichia coli*, and two did not specify the bacterial origin of LPS. Furthermore, the mean LPS dose was 294 ng/mL (range, 0.5–1,000 ng/mL), and the mean stimulation time was 11 h (range, 3–24 h). The standardized mean difference for eight of the nine included studies suggested that diminished TNF production at sepsis onset may be associated with increased mortality (Fig 2A), although the large variance in the confidence of these estimates and the heterogeneous study design precluded meta-analysis of the data.

Of the 17 initially identified studies, 47% were deemed to be of moderate quality and 41% were deemed to be of high quality according to the NOS score. Although many of these observational studies showcased clear clinical outcomes, significant variation was found

in the selection domain, particularly regarding consecutive patient enrollment, multiple investigators' involvement in patient selection, and the masking to each other's eligibility decisions. Regarding the comparability between cohorts (survivors vs nonsurvivors), most studies did not evaluate or report potential covariates, like severity of illness scores, at the point of patient recruitment.

To evaluate potential publication bias, we used the *meta* statistical package to infer hypothetical missing studies necessary for a less biased adjusted estimate. This could account for studies not published because of negative results, those that could not be performed because of low study feasibility (eg, large clinical studies), or those overlooked in the systematic review because of language barriers or mistaken exclusions. The funnel plot suggested three such studies, or a publication bias of approximately 18% considering the 17 studies that were included in the analysis (Fig 3A).

Association Between LPS-Induced TNF Concentration and Survival in Established Sepsis

Five studies reported LPS-induced TNF concentrations during established sepsis, yielding a total of 239 clinical observations (Fig 2B). Four of these five studies involved stimulation of whole blood with LPS, and one study described stimulation of peripheral blood mononuclear cell with LPS. Three studies used LPS derived from *S abortusequi*, one study used LPS derived from *E coli*, and one study did not specify the bacterial origin of LPS. Mean LPS dose was 201 ng/mL (range, 0.5–1,000 ng/mL), and mean duration of stimulation was 11 h (range, 4–24 h). As in the case of sepsis onset, the data suggested that diminished TNF production during established sepsis may be associated with increased mortality, although study heterogeneity precluded pooling of the data for meta-analysis. Funnel plot analysis suggested a publication bias of up to 40%, given that two imputed studies would be required to restore plot symmetry.

Association Between LPS-Induced TNF Concentration and Secondary Outcomes

Of the 17 included studies, 14 studies reported both mortality and nonmortality outcomes, whereas three studies reported nonmortality outcomes alone (Table 1).[49–51] Key study findings are summarized in Table 2. Heterogeneity in study design and in the secondary clinical outcomes that were described did not permit synthesis of these data. However, studies identified by the present review suggest an association between decreased stimulated cytokine production and persistent[39] or worsening[49,52] organ dysfunction within the first week of sepsis onset. The reported relationship between stimulated cytokine production and the risk of nosocomial infection was inconsistent between studies.[8,37,47]

Discussion

The diverse immune responses in patients with sepsis complicate the comparison of diagnostic and therapeutic trial results, making the development of innovative treatments a challenge.[53] This complexity has led to interest in functional immune phenotyping, a technique that quickly evaluates immune function to predict outcomes in patients with sepsis.[54] LPS, which has been used as an immune stimulant for many years, is a favored tool in this approach. However, inconsistent study methodologies over the past 25 years

have impacted its effectiveness. This systematic review, which sought to establish a connection between LPS-induced TNF production and mortality, was hampered by these inconsistencies, making a comprehensive meta-analysis unfeasible.

Evolving sepsis definitions also pose challenges to this field of research, especially when examining long-spanning literature. Our understanding of sepsis has evolved, resulting in shifts in diagnostic benchmarks. For instance, although Sepsis-1 was based on the Systemic Inflammatory Response Syndrome criteria,[27] Sepsis-3 transitioned to emphasize organ dysfunction via the Sequential Organ Failure Assessment score.[29] This evolution suggests a potential misalignment in patient inclusion across different study periods, making the comparison of patient populations described in different studies challenging. Moreover, given the rapid progression of sepsis, particularly among critically ill patients, inconsistent timing in blood sample collection may amplify the diagnostic inconsistencies arising from shifting definitions of sepsis onset. To mitigate the potential confounding effect of variable blood sample collection times, especially in the absence of a sepsis-specific biomarker, future research should aim to standardize blood collection protocols based on the timing of onset of sepsis-related symptoms, rather than the timing of hospital or ICU admission for sepsis. In our current analysis, we categorized the data into two broad types for more straightforward comparative study: sepsis onset and established sepsis.

Heterogeneity in the LPS-based assay protocol could exacerbate observed study differences. Approximately one-half of the studies used LPS derived from *S abortusequi*. Despite a universal incubation temperature for patient blood with LPS at 37 °C, a striking variation existed in LPS dosages and exposure durations. Prevailing wisdom posits that cytokine production after stimulation hinges on dosage and duration.[55] As the ex vivo immune response evolves, diverse cell groups, notably monocytes and macrophages, begin TNF production, reaching a peak in 4 to 6 h.[56] Dendritic cells and natural killer cells also join this production within the first 24 h after LPS stimulation.[57,58] TNF kinetics become more intricate with extended LPS stimulation, showing potential for immune cell tolerance,[59] secretome alterations,[60] and cellular apoptosis.[61] Thus, it is essential to standardize the stimulation timing for a larger cohort of patients with sepsis.

The infectious cause of sepsis also may influence the observed outcomes. Of the 17 identified studies, 10 studies did not provide microbial data, and among those that did, the prevalence of gram-negative organisms ranged between 11% and 51%. A prior exposure to endogenous LPS in gram-negative bacteria-induced sepsis might prime the patient's immune cells, impacting the reaction to subsequent ex vivo LPS stimulation (a phenomenon known as *endotoxin tolerance*).[62] Gram-positive bacteria might elicit a stronger response, unencumbered by previous LPS exposure.[63] Thus, LPS-induced TNF levels must be interpreted in light of the infectious origin, or at the very least, its potential influence should be acknowledged.

Regarding clinical outcomes, 30-day mortality rates might not entirely encapsulate sepsis-related immune dysfunction. Even slight immune impairments can lead to secondary infections, not necessarily fatal, but potentially extending hospital stays or prompting hospital readmission. This highlights the analytical limitation of using mortality as a primary

outcome measure. Despite inherent drawbacks, mortality was our primary choice, given its prevalent use in clinical studies and its unequivocal reliability, especially amidst varying study designs. Alternative outcomes like nosocomial infection rates or duration of critical illness may provide deeper immunologic insight, but come with their own set of limitations.

This study also highlighted the varied definitions of immune paralysis and alternative LPS-stimulated cytokines of interest across studies. Acknowledgment of the role of T-cell dysfunction in sepsis is growing.[14,15] Consequently, some studies, such as that by Mazer et al,[43] used T-cell stimulants, finding suppressed interferon γ production linked to sepsis mortality. Our findings suggest that future immune phenotyping endeavors might benefit from a more comprehensive panel of immune stimulants that probe both innate and adaptive immune mechanisms.

Transcriptomic research has deepened our knowledge of the varied immune functions in sepsis. One notable sepsis subclass, commonly referred to as SRS1 or endotype A, involves repression of key driver genes of the innate and adaptive immune systems and results in particularly poor patient outcomes.[64–66] However, analyzing gene expression is expensive, time intensive, and laborious. This becomes even more challenging given the fast-paced and life-threatening nature of sepsis.

Using point-of-care measurement for rapid functional immune profiling may help clinicians to apply transcriptomic discoveries to enhance patient care. For instance, by detecting patients with immune paralysis in real time, clinicians could consider giving immune-enhancing therapies to prevent sepsis-related complications and even death. The Interleukin-7 Restores Lymphocytes In Septic Shock (IRIS-7) trial, the first to test immunoadjuvant therapy for patients with sepsis, showed that recombinant IL-7 counteracted the significant loss of CD3 and CD8 immune cells, a primary characteristic of sepsis contributing to its morbidity and mortality.[17] Moreover, functional immune profiling could shed light on the longstanding discussion about benefit of corticosteroids in treating sepsis.[67]

To realize these ambitious therapeutic objectives, the current systematic review emphasizes the pressing need for a unified definition of immunoparalysis through standardized research protocols. This encompasses uniform dosages of immune stimulants, consistent incubation conditions, clear patient recruitment methodologies, and the adoption of standardized clinical outcomes. Implementing these standards could enhance the robustness of conclusions from smaller clinical trials and could facilitate more effective data synthesis across multiple centers.

Interpretation

Future research focusing on LPS-induced TNF production for functional immune profiling should prioritize characterization of the microbial source of sepsis (ie, gram-negative infection vs gram-positive infection) as well as protocol standardization with respect to the definition of immunoparalysis. Adopting uniform nonmortality outcomes definitions, such as that used for nosocomial infections, will be invaluable. Such standardizations would

pave the way for acquiring consistent data from expansive patient groups, spotlighting rarer adverse outcomes, but those with impact, in patients with sepsis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS:

LPS	lipopolysaccharide
NOS	Newcastle-Ottawa scale
TNF	tumor necrosis factor

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Take-home Points

Study Question:

Does lipopolysaccharide-induced tumor necrosis factor (TNF) production from the blood of patients with sepsis predict mortality?

Results:

Selected studies indicated that lower lipopolysaccharide-induced TNF production may correlate with higher mortality in sepsis, but methodologic variations and biases limited definitive conclusions.

Interpretation:

Streamlined research methodologies, including standardized definitions of immunoparalysis and consistent procedures for ex vivo blood stimulation, are vital for functional immune profiling in sepsis.

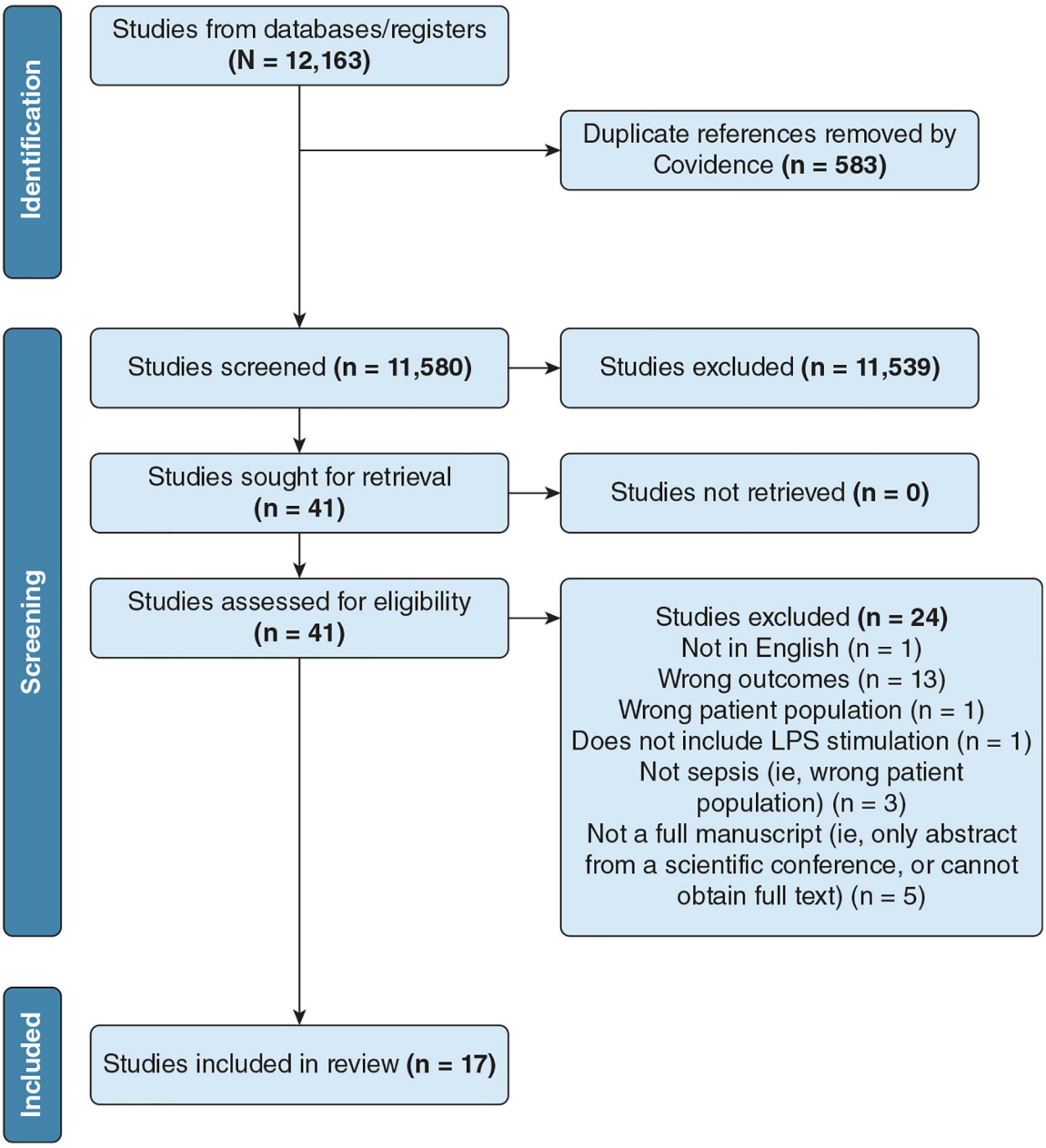

Figure 1 –.

Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart depicting the study selection process. LPS = lipopolysaccharide.

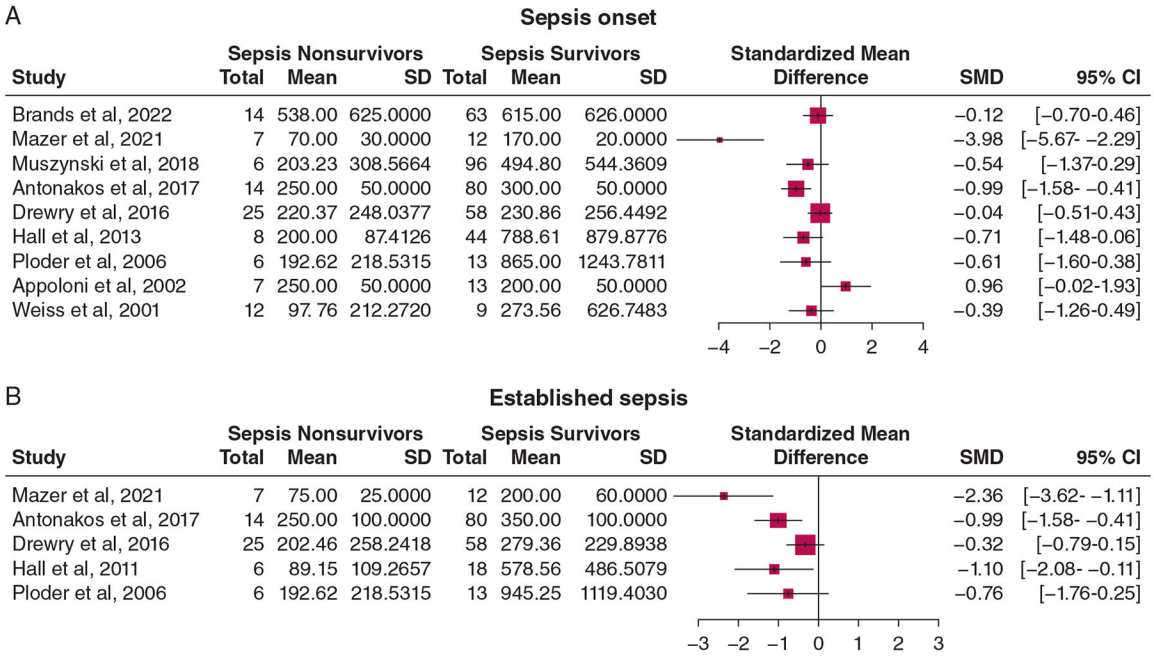

Figure 2 –.

A, B, Forest plots illustrating the correlation between lipopolysaccharide-induced tumor necrosis factor concentrations (measured in picograms per milliliter) and mortality at the time of sepsis onset (A) and during established sepsis (B). SMD = standardized mean difference.

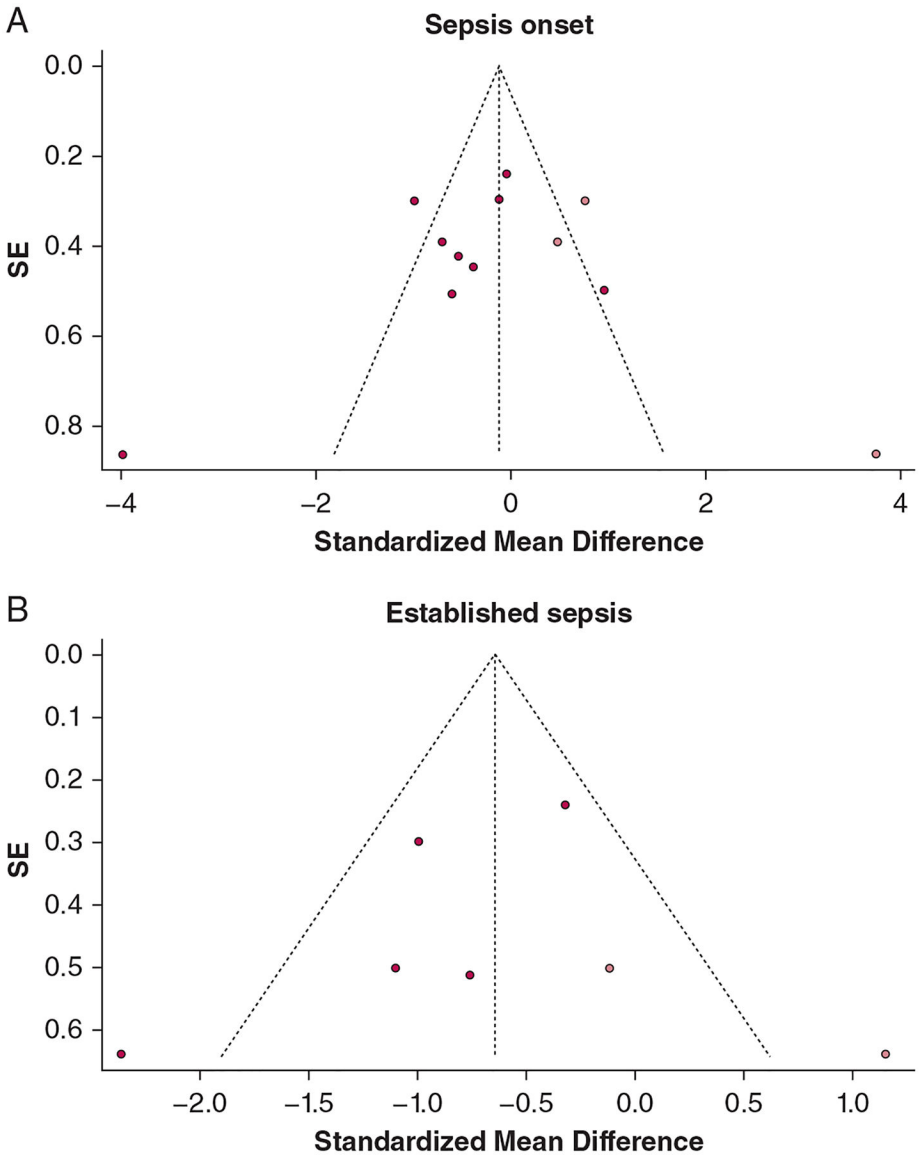

Figure 3 –.

A, B, Funnel plots illustrating the distribution of study effect sizes in relationship to their SEs, used to evaluate potential publication bias: studies describing the relationship between lipopolysaccharide-induced tumor necrosis factor concentrations and mortality at the time of sepsis onset (A) and during established sepsis (B). Filled circles represent observed studies with real data points, and hollow circles represent hypothetical missing studies that would need to be included to give an adjusted estimate that is potentially less biased than the original.

TABLE 1]

Stimulants, Cytokines, and Clinical Outcomes

Authors (Year)	Study Population	Microbial Pathogens	Time of Blood Sampling (Relative to Clinical Diagnosis of Sepsis)	LPS Exposure Conditions (Bacterial Strain, Dose/10^6 Cells, Incubation Time)	Primary Cytokine Measured	Primary Clinical Outcome Predicted by the Study	Secondary Clinical Outcome Measured
Brands et al[42] (2022)	Critically ill adults with sepsis (n = 77)	NR	9 am on first day of admission to ICU	*Ecoli* O111:B4, 100 ng/mL, 3 h	TNF	ICU length of stay	ICU mortality
Mazer et al et al[43] (2021)	Critically ill adults with sepsis (n = 19), critically ill adults without sepsis (n = 6), healthy control participants (n = 20)	26% gram-positive, 16% gram-negative, 16% mixed	24–48 h	S *abortusequi*, 2.5 ng/mL, 18–22 h	TNF	In-hospital mortality	NA
Maddux et al[39] (2019)	Critically ill adults with sepsis (n = 19), critically ill adults without sepsis (n = 6), healthy control participants (n = 20)	48% gram-positive, 33% gram-negative	Day 1 of ICU admission, day 4 (± 24 h) and day 6 (± 24 h)	*E coli* (strain not specified), 500 pg/mL, 4 h	TNF	Organ recovery (decrease SOFA score)	In-hospital mortality
Nesseler et al[49] (2019)	Critically ill adults with severe sepsis (n = 12) or septic shock (n = 37), healthy control participants (n = 23)	61% gram-positive, 51% gram-negative [sic]	< 24 h of ICU admission, and day 7	E *coli* O55:B5, 10 μg/mL, 24 h	IL-10	Organ recovery (SOFA score < 2)	NA
Muszynski et al[52] (2018)	Critically ill children with severe sepsis (n = 23) or septic shock (n = 79), healthy control participants (n = 35)	28% gram-positive, 11% gram-negative	< 48 h	*S abortusequi*, 500 pg/mL, 5 h	TNF	Organ recovery	In-hospital mortality
Antonakos et al[48] (2017)	Adults with sepsis (n = 95), of whom 26% had “failing organs on enrollment”	29% gram-negative	< 24 h, and days 3, 7, and 10	*Ecoli* O55:B5, 10 ng/mL, 24/48 h	IL-10	28-d mortality	NA
Fang et al[38] (2017)	Critically ill adults with sepsis (n = 151)	NR	Day 1 of ICU admission	Not specified, 100 ng/mL, 4 h	G-CSF	28-d mortality	NA
Drewry et al[47] (2016)	Critically ill adults with severe sepsis (n = 85)	NR	Days 1–2, days 3–4, days 6–8	*S abortusequi*, 0.5 ng/mL, 4 h	TNF	28-day mortality	Nosocomial infection
van Vught et al[51] (2015)	Critically ill adults with severe sepsis (n = 85)	NR	9:00 AM of the first morning after ICU admission	*Ecoli* O111:B4, 100 ng/mL, 3 h	TNF	ICU-acquired infection	NA
Hall et al[46] (2013)	Critically ill children with influenza sepsis (n = 52), healthy control participants (n = 21)	Influenza A (39%), with 26% gram-positive coinfection	< 72 h of ICU admission	*S abortusequi*, 500 pg/mL, 4 h	TNF	In-hospital mortality	Coinfection at time of study enrollment
Saha et al[50] (2012)	Adults with sepsis (n = 10) and septic shock (n = 11), healthy control participants (n = 19)	29% gram-positive, 24% gram-negative, 29% mixed	< 24 h, days 5–7, and days 14–16	*Salmonella Minnesota* Re 595, 100 μg/mL, 1.5 h	Superoxide	Organ recovery (decrease in APACHE II score ≥ 6)	NA

Authors (Year)	Study Population	Microbial Pathogens	Time of Blood Sampling (Relative to Clinical Diagnosis of Sepsis)	LPS Exposure Conditions (Bacterial Strain, Dose/10^6 Cells, Incubation Time)	Primary Cytokine Measured	Primary Clinical Outcome Predicted by the Study	Secondary Clinical Outcome Measured
Hall et al[37] (2011)	Critically ill children (n = 70) of whom 30 patients had a primary diagnosis of sepsis, and healthy control participants (n = 8)	NR	Days 3, 7, and 14	*S abortusequi*, 500 pg/mL, 4 h	TNF	Nosocomial infection	ICU mortality
Ploder et al[44] (2006)	Critically ill adults with multiple trauma (n = 21) of whom 19 patients had sepsis, and healthy control participants (n = 7)	NR	< 24 h after ICU admission, and daily thereafter until 14 d	Not specified, 500 pg/mL, 4 h	TNF	In-hospital mortality	NA
Appoloni et al[41] (2002)	Critically ill adults with septic shock (n = 21)	NR	NR	*Ecoli* O26:B6, 0.2 and 1 ng/mL, 16 h	TNF	ICU mortality	NA
Weiss et al[40] (2001)	Critically ill adults with septic shock (n = 21), healthy control participants (n = 11)	NR	Daily while in ICU	S *abortusequi*, 10 μg/mL, 24 h	TNF	ICU mortality	NA
Adib-Conquy et al[36] (2000)	Critically ill adults with severe sepsis (n = 19) or major trauma (n = 13), healthy control participants (n = 13)	NR	Day 1 of ICU admission, and day 7	*Ecoli* O111:B4, 1 μg/mL, 1 h	NF-κB	7-d mortality	NA
Adamik et al[25] (1997)	Critically ill adults with sepsis (n = 53), healthy control participants (n = 14)	NR	Day 1 of ICU admission, and 1, 2, and 5 d later	Not specified, 10 μg/mL, 24 h	TNF	Mortality (time to outcome not specified)	NA

APACHE = Acute Physiology and Chronic Health Evaluation; LPS = lipopolysaccharide; NA = not applicable; NF-κB = nuclear factor-κB; NR = not reported; SOFA = Sequential Organ Failure Assessment; TNF = tumor necrosis factor.

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TABLE 2]

Summary of Main Findings and Bias Assessment Score

		Newcastle-Ottawa Score for Risk of Bias Assessment			
Authors (Year)	**Summary of Main Findings**	**Selection (Maximum 4)**	**Comparability (Maximum 2)**	**Outcomes (Maximum 3)**	**Overall Score (Maximum 9)**
Brands et al[42] (2022)	Stimulated TNF production was not associated with ICU length of stay or ICU mortality in critically ill adults with sepsis.	★★★★	★★	★★★	★★★★★★★★★
Mazer et al[43] (2021)	Decreased concentrations of stimulated TNF production were associated with increased mortality in critically ill adults with sepsis.	★★☆☆	★★	★★★	★★★★★★★★☆
Maddux et al[39] (2019)	Stimulated TNF production was not associated with organ recovery at day 4, although TNF production at day 6 was significantly lower in patients who did not recover organ function at day 4, in critically ill adults with sepsis.	★☆☆☆	★☆	★★½	★★★★½ ☆☆☆☆
Nesseler et al[49] (2019)	A low ratio of stimulated to unstimulated IL-10 production was associated with decreased organ dysfunction at day 7 in critically ill adults with sepsis.	★½ ☆☆	☆☆	★★½	★★★½ ☆☆☆☆☆
Muszynski et al[52] (2018)	Stimulated TNF production of < 186 pg/mL in critically ill children with sepsis was associated with new or progressive multiorgan dysfunction syndrome, or both.	★☆☆☆	★☆	★★☆	★★★★☆☆☆☆☆
Antonakos et al[48] (2017)	Decreased concentrations of stimulated TNF production were associated with increased mortality in adults with sepsis.	★★½ ☆	★☆	★★★	★★★★★★½ ☆☆
Fang et al[38] (2017)	Granulocyte colony stimulating factor elevation ratio (cytokine level after LPS stimulation divided by cytokine level before LPS stimulation) was not associated with 28-d mortality in critically ill adults with sepsis.	★★★☆	★★	★★★	★★★★★★★★☆
Drewry et al[47] (2016)	Stimulated TNF production was not associated with mortality or nosocomial infections at any measured time point in critically ill adults with sepsis.	★★☆☆	★★	★★★	★★★★★★★★☆
van Vught et al[51] (2015)	Stimulated TNF production was not associated with ICU-acquired infections in critically ill adults with sepsis.	★★★☆	★★	★★½	★★★★★★★½ ☆
Hall et al[46] (2013)	Decreased concentrations of stimulated TNF production were associated with increased mortality in critically ill children with influenza sepsis. Reduced TNF production conferred a higher risk of *Staphylococcus aureus* coinfection at the time of influenza diagnosis as compared with patients without bacterial coinfection or those with non-*S aureus* coinfection.	★☆☆☆	★★	★★★	★★★★★★★☆☆
Saha et al[50] (2012)	Stimulated superoxide production was not compared directly between adults with sepsis who recovered and who did not recover organ function.	★★☆☆	★☆	★½ ☆	★★★★½ ☆☆☆☆
Hall et al[37] (2011)	TNF concentration of < 200 pg/mL (ie, immunoparalysis) throughout 7 d after positive microbial culture findings was associated with persistent nosocomial infection, whereas recovery of > 200 pg/mL was associated with resolution of infection.	★☆☆☆	½ ☆	★★☆	★★★☆☆☆☆
Ploder et al[44] (2006)	Decreased concentrations of stimulated TNF production were associated with increased mortality in critically ill adults with sepsis.	★★☆☆	★☆	★★★	★★★★★★★☆☆
Appoloni et al[41] (2002)	Survivors of critical illness secondary to sepsis produced higher concentrations of TNF after delayed exposure to LPS (ie, 16 h resting period followed by 8 h LPS stimulation) as compared with nonsurvivors.	★★☆☆	★☆	★★★	★★★★★★★☆☆

		Newcastle-Ottawa Score for Risk of Bias Assessment			
Authors (Year)	**Summary of Main Findings**	**Selection (Maximum 4)**	**Comparability (Maximum 2)**	**Outcomes (Maximum 3)**	**Overall Score (Maximum 9)**
Weiss et al[40] (2001)	Stimulated TNF production was not associated with mortality in critically ill adults with septic shock.	★★☆☆	½ ☆	★★★	★★★★★½ ☆☆☆
Adib-Conquy et al[36] (2000)	Survivors of critical illness secondary to sepsis showed a lower ex vivo nuclear expression of NF-κB before and after LPS stimulation.	★★★☆	★☆	★★½	★★★★★★★☆☆
Adamik et al[25] (1997)	Stimulated TNF production was not associated with mortality in critically ill adults with sepsis.	★★☆	★☆	★★½	★★★★★★½ ☆☆

LPS = lipopolysaccharide; NF-κB = nuclear factor-κB; TNF = tumor necrosis factor.