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INPLASY

Treating sepsis by targeting neutrophil extracellular traps: a protocol for a meta-analysis and systematic review

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ADMINISTRATIVE INFORMATION

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Review Stage at time of this submission - Formal screening of search results against eligibility criteria.

Conflicts of interest - None declared.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 2 September 2025 and was last updated on 2 September 2025.

INTRODUCTION

Review question / Objective Primary Objective: Pooled septic animal studies investigating the use of therapies to improve outcomes via the modulation of NETs formation. Secondary Objective: Demonstrate the need to reduce research waste and uncover reasons for the reported low translatability from animal studies to clinical trials in sepsis.

Sepsis is a life-threatening condition caused by a dysregulated host response to infection. This condition remains a leading cause of global mortality, with limited effective therapies. Recent research has suggested that imbalanced neutrophil extracellular traps (NETs) are implicated in worsening sepsis outcomes. This protocol outlines a systematic review and meta-analysis of animal studies investigating therapeutic strategies that target NETs in sepsis models. We will evaluate interventions that modulate NETs formation or degradation and assess outcomes such as survival and organ protection. Our findings aim to reduce research waste, strengthen translational relevance, and inform future therapeutic development for sepsis.

Rationale Sepsis is a life-threatening condition resulting in organ dysfunction via a dysregulated host response to infection, as defined by the Third International Consensus Definitions for Sepsis and Septic Shock [1]. In 2017, sepsis accounted for nearly 50 million cases and 11 million deaths worldwide, making it a major global healthcare challenge [2]. Despite its widespread impact, there is still no single treatment proven to reduce mortality significantly. Current healthcare methods include controlling the source of infection, administering broad-spectrum antibiotics with fluids, and providing organ supportive care [3]. Recently, research has shown that dysregulated neutrophil extracellular traps (NETs) formation and

clearance may increase the severity of sepsis [4]. However, there is a lack of clarity regarding the efficacy of these treatment strategies in animal models of sepsis.

While sepsis is a complex syndrome, this review will look specifically at the effect of NETs on the severity of sepsis in animal models. NETs are extracellular fibers from neutrophils that contain nuclear material, including histones and granular proteins [5]. Fuchs et al demonstrated that they form via the NETosis pathway to trap and neutralize pathogens [6]. However, dysregulated NETs formation and clearance may be increasing the severity of sepsis, specifically by increasing inflammation, causing tissue damage, and promoting thrombosis [4]. This discovery has encouraged researchers to pursue more effective therapies by modulating NETs formation or degradation [7].

Considering the growing interest in NETs as therapeutic targets, we will conduct a systematic review and meta-analysis of animal studies evaluating NET-targeted interventions for sepsis [8]. By synthesizing and critically appraising the available evidence, this work may provide an example of a methodological evaluation of animal research, ultimately improving the translatability of sepsis research into clinical practice.

Condition being studied We will conduct a systematic review and meta-analysis of studies reporting the use of therapeutics targeting NETs in wild-type animal models of sepsis. We will include both randomized and nonrandomized study types, specifically animal studies using wild-type models with a control group. No studies will be excluded if they meet the inclusion criteria.
The Third International Consensus Definitions Task Force defined sepsis as life-threatening organ dysfunction caused by a dysregulated host response to infection [1]. In clinical practice, the Sequential Organ Failure Assessment (SOFA) score is implemented to determine the severity of sepsis and associated mortality rate. However, using the SOFA score is quite difficult in animal models. For this study, we will use the sepsis intervention model to define sepsis, i.e., an exogenous toxin, administering a pathogen, or enabling the release of endogenous pathogens. Specifically, we will focus on bacterial infusion, cecal ligation and puncture, endotoxemia, cecal slurry injection, and purified lipopolysaccharide (LPS) infusion [8].
To quantify NETs, we will rely on specific universal biomarkers including nucleosomes containing histone H3.1, myeloperoxidase (MPO), neutrophil elastase (NE), and cell-free DNA (cfDNA) [10,11,12]. These biomarkers are extensively studied and have been shown to quantify NETs in multiple studies [13]. Researchers have indicated that histone H3.1 is released during cell death and is critical for neutrophil activation. More specifically, citrullinated H3, which is a NET-specific modification by PAD4, has been demonstrated to be an early detection marker of sepsis [10]. MPO and NE are neutrophil granule enzymes involved in NETosis and thus used in methods that measure MPO-DNA and NE-DNA complexes as a marker of systemic inflammation [11]. Another biomarker is cfDNA, which is released from NETs and damaged cells and is useful in the early detection of sepsis [12].

METHODS

Search strategy We generated the search strategy using the advanced search string on PubMed and Scopus. The result of the literature search will be exported to the reference management system EndNote (EndNote v.20, Clarivate, Philadelphia, PA, USA) for duplicate removal. The search strategy and literature search will be peer reviewed by C.F. and B.R.

19.1. Search String
19.1.1. PubMed search string is the following:
("sepsis"[MeSH Terms] OR ("sepsis"[Title/Abstract] OR "septicemia"[Title/Abstract] OR "septic shock"[Title/Abstract] OR "bacteremia"[Title/Abstract])) AND ("extracellular traps"[MeSH Terms] OR ("neutrophil extracellular traps"[Title/Abstract] OR "NETs"[Title/Abstract])) AND ("lipopolysaccharides"[MeSH Terms] OR ("Lipopolysaccharide"[Title/Abstract] OR "LPS"[Title/Abstract])) AND ("animal models"[Title/Abstract] OR ("animal model"[Title/Abstract] OR "experimental animal models"[Title/Abstract]))
19.1.2. Scopus search string is the following:
((KEY (sepsis)) OR ((TITLE-ABS-KEY (sepsis) OR TITLE-ABS-KEY (septicemia) OR TITLE-ABS-KEY (septic AND shock) OR TITLE-ABS-KEY (bacteremia)))) AND ((KEY (neutrophil AND extracellular AND traps)) OR ((TITLE-ABS-KEY (neutrophil AND extracellular AND traps) OR TITLE-ABS-KEY (nets)))) AND ((KEY (lipopolysaccharide)) OR ((TITLE-ABS-KEY (lipopolysaccharide) OR TITLE-ABS-KEY (lps) OR TITLE-ABS-KEY (live AND bacteria)))) AND ((KEY (animal AND model)) OR ((TITLE-ABS-KEY (animal AND model) OR TITLE-ABS-KEY (experimental AND animal AND models) OR TITLE-ABS-KEY (wild AND type AND animal)))).

Participant or population We will include studies that use sepsis animal models of all sexes and

species. Specifically, we will focus on bacterial infusion, cecal ligation and puncture, endotoxemia, injection of a cecal slurry, and infusion of purified lipopolysaccharide (LPS). We will exclude knockout models, ex vivo, in vitro, and in silico models.

Intervention Of interest are interventions addressing NETs formation and clearance. We will also consider other types of interventions, such as homeopathic methods, subject to what exists in the literature.

We will classify NETs therapies described in studies according to the following broad categories:
• Homeopathic method (Re-Du-Ning injection)
• Agonist of NETs (Hydralazine and procainamide)
• Inhibitor of NETs (AFM-30a and GSK199)
• Degradation of NETs (DNASE1)
• Antibiotics(meropenem).

Comparator We will include all wild-type animals that model sepsis and undergo no therapeutic treatment as our control. If the control meets our criteria for the participants, no comparator will be excluded.

Study designs to be included Both randomized and nonrandomized study types will be included.

Eligibility criteria
Inclusion criteria are the following:
• All animals (all species/sex)
• Sepsis model
• Therapy targeting NETs (all doses/timing)
• Non-exposed to therapy control group
• Must quantify NETs
• Must quantify improvement (Reduces proinflammatory cytokines, reduces damage in histology and pathology of tissue, increases survival, decreases organ injury markers)
Exclusion criteria are the following:
• Human
• Non-wild-type animals
• In vitro/in silico studies
• No control group in a wild-type animal
• Reviews/conference abstracts
• Non-English papers
• Over 10 years old.

Information sources Literature search strategies will be created using medical subject headings (MeSH) and Title/Abstract words related to sepsis animal models with a focus on NETs. We will perform the literature review in two different electronic search engines (PubMed and Scopus) for records between January 1, 2015, and May 31, 2025.

The International Prospective Register of Systematic Reviews (PROSPERO) has been searched for ongoing or recently completed systematic reviews. We did not find an ongoing or completed systematic review on the topic of novel therapeutics for sepsis in association with NETs in animal models; therefore, we registered this protocol in PROSPERO 2025 (Protocol ID: CRD420251124339).

Main outcome(s) Primary outcomes: NETs measurements, Outcome improvement measurements (Reducing proinflammatory cytokines, reducing damage in histology and pathology of tissue, increasing survival, decreasing organ injury markers). Excluded papers' outcomes: Do not demonstrate that the therapy affected the NETs quantities, which in turn improved the outcome of the animal.

Data management The remaining titles after duplicate removal will be exported to CAMARADES Software (Preclinical Systematic Reviews & Meta-Analysis Wiki, (March 2025), CAMARADES Berlin, QUEST-BIH Charité. Accessed from: https://www.CAMARADES.de). One review author (C.F.) will independently perform a blinded screening process of the titles and abstracts of the retrieved articles for potential eligibility. Any disagreement or doubt about potential eligibility will be resolved by discussion with B.R., S.T., and S.F.

Quality assessment / Risk of bias analysis Two authors (C.F. and B.R.) will independently assess the risk of bias of the included articles by using SYRCLE's Risk of Bias tool, which is a tailored version of the Cochrane RoB tool for bias in animal intervention studies [14].

We will follow the SYRCLE RoB question to assign a low, high, or unclear risk. S.F. will be consulted if disagreements occur. Our assessment will be presented in a table as a summary of the results.

Strategy of data synthesis After the blinded screening process, we expect the data to be heterogeneous in terms of the type of therapy, the NETs quantification method, and the improvement quantification method. Thus, we will synthesize the data based on the Cochrane method of vote counting based on the direction of effect. As all these studies are in a preclinical animal model, no consistent effects or data have been reported. Hence, we will create a standardized binary metric,

first determine an increase or decrease of NETs, then determine the benefit or harm based on the outcome of the therapy. We will tabulate the binary metric alongside any effect estimates. Then use the table to derive the harvest and effect direction plots. To run a statistical analysis, we will run a binomial probability test, or equivalently, the sign test [15]. If the heterogeneity is too extreme, we will run the standardized binary metric on subgroups, such as the type of therapy and/or the improvement quantification.

Type of Therapy
Homeopathic method 0
Agonist of NETs 1
Inhibitor of NETs 2
Degradation of NETs 3
Antibiotics 4
NETs Quantification
Outcome Binary Metric
Reduces NETs 1= Yes
0=No
Improvement Quantification
Outcome Binary Metric
Reduces proinflammatory cytokines 1= Yes
0=No
Reduces damage in histology and pathology of tissue 1= Yes
0=No
Increase survival 1= Yes
0=No
Decreases organ injury markers 1= Yes
0=No.

Subgroup analysis If the heterogeneity is too extreme, we will run the standardized binary metric on subgroups, such as the type of therapy and/or the improvement quantification.

Sensitivity analysis The analysis has yet to be determined.

Language restriction English.

Country(ies) involved Sweden, Germany.

Keywords Sepsis; Animal model; Neutrophil extracellular traps.

Dissemination plans Sepsis is a leading cause of death worldwide, and despite decades of research, consistent and effective treatments remain limited. Some studies have focused on the immune system, particularly the dysregulation of NETs. Similarly, numerous preclinical animal studies have explored novel NETs therapies, but, to our knowledge, no systematic review has critically analyzed sepsis therapies targeting NETs in animal models. This review aims to summarize existing research, to offer new insights into sepsis treatment, and to guide future research directions.

One key benefit of this review is its alignment with the principles of the 3Rs, specifically, the reduction and refinement of animal use. By synthesizing data from multiple studies, we aim to strengthen the statistical evidence supporting specific therapies. We hope our findings will improve information accessibility and make it easier for future researchers to build upon past discoveries. Given the variability in both animal models and sepsis itself, we also hope this review can guide the design of future studies, encouraging alignment with established standards and promoting greater consistency in preclinical sepsis research.

However, achieving these benefits may be challenging due to heterogeneity among the studies, particularly in terms of therapeutic targets and methods of analysis. This variability may limit the feasibility of conducting a comprehensive meta-analysis. To address this, we will first develop a predefined binary metric that can be universally applied across all included studies. Secondly, we will perform subgroup analyses based on therapy type and the specific outcome measures used to assess improvement.

Contributions of each author

Author 1 - Carley Fowler - Author 1 drafted this protocol. She will screen the studies, extract the data, analyze the data, and write the manuscript.
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Author 2 - Bahareh Razmand - Author 2 will screen the studies and pull the data. She helped with the search strings.
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