**1.0 Introduction**

***1.1 Problem Statement***

Annually, approximately 400,000 Americans and 40,000 Canadians undergo cardiac surgery.[1,2]  This critical intervention carries significant postoperative risks, including infections that may lead to sepsis.[3-5] The increasing incidence and high mortality rates, reaching up to 33%, from septic shock underscore the urgency to identify individuals at risk. Notably, the risk of postoperative sepsis (POS) is substantially elevated.[6-11] However, current clinical indicators for predicting POS are insufficient. Our study aims to revolutionize patient management by identifying gene networks and biomarkers predictive of sepsis following cardiac surgery.[10]

***1.2 Proposed Solutions***

We propose an innovative approach, conceptualizing sepsis as a complex syndrome modulated by networks of interacting genes (refer to Figure 1). [12,13] Utilizing next-generation sequencing (NGS), we plan to identify relevant genes and proteins, followed by thorough validation. Our focus is on analyzing gene networks using techniques such as weighted gene co-expression network analysis (WGCNA) for module identification.[16] This multi-gene strategy is further enhanced by utilizing the Gene Expression Omnibus (GEO) database for comprehensive genetic insights into sepsis.[14,15]



Figure 1. Part of Sepsis Mechanism Overview. This figure illustrates key aspects of sepsis immunity and potential therapeutic targets, adapted from Wiersinga WJ's work, entitled "Immunopathophysiology of Human Sepsis" in the journal of *EBioMedicine*.

***1.3 Methodology for Validation***

Validation will be conducted using data from the international Cardiac Surgery Biobank cohort. Our goal is to establish a correlation between specific gene networks and the incidence of POS, advancing beyond traditional polygenic risk scores towards a nuanced gene network-based risk assessment.[15]

***1.4 Broader Implications***

Supported by Heart and Stroke, our research focuses on in-depth gene network analysis within the Cardiac Surgery Biobank cohort. The insights obtained are expected to significantly enhance our understanding of the genetic foundations of adverse outcomes post-cardiac surgery.

**2.0 Study Hypotheses**

* Patients with POS following cardiac surgery will exhibit distinctive gene network patterns linked to sepsis.
* A correlation is expected between the expression of these gene networks and the incidence of sepsis within one-month post-surgery or with gene detection potentially predicting sepsis occurrence on a weekly basis.
* The accuracy of these gene networks in predicting sepsis will increase as the onset of sepsis approaches.

**3.0 Objectives**

* To identify and compare gene network patterns in patients with and without new-onset POS post-cardiac surgery.
* To evaluate the incremental predictive value of these gene networks over traditional clinical assessments for POS prediction.
* To perform comprehensive genetic testing at critical time points, from pre-surgery to the development of postoperative sepsis.

**4.0 Existing Knowledge**

***4.1 Challenges in POS Management***

The global increase in cardiovascular diseases has highlighted the critical need for effective management of sepsis, characterized by systemic inflammation and organ dysfunction, following cardiac surgery. Prompt detection and timely intervention are pivotal in improving patient outcomes.[4,6,17]

***4.2 Need for Enhanced POS Prediction***

Emerging evidence suggests that gene network analysis could significantly improve predictive accuracy for POS, thereby offering a novel dimension in preemptive patient treatment strategies.

***5.0 Methodological Approach***

Our approach encompasses a detailed analysis of the VISION Cardiac Surgery cohort, which includes 3,737 patients from diverse geographical locations spanning 12 countries. Our genetic analyses will adhere to rigorous standards, followed by a comprehensive data analysis process utilizing R software. This includes meticulous preprocessing of raw NGS data, in-depth analysis of RNAseq data employing the edgeR package, differential gene analysis, and sophisticated enrichment of the dataset with both GO and KEGG terms.

***6.0 Study Design***

We will stratify participants based on their sepsis history to compare gene network patterns. Advanced statistical methods and machine learning algorithms will be utilized for differential expression analysis and model optimization.

**7.0 Justification for Study Design**

***7.1 Cohort Utilization***

Employing the VISION Cardiac Surgery Biobank cohort, with its international scope and comprehensive data collection, is ideal for exploring the relationship between gene networks and POS.

***7.2 Limitations of Other Designs***

The challenges with the VISION Heart Biobank include incomplete longitudinal data and the need to address epigenetic factors like DNA methylation, which are pivotal in sepsis pathogenesis.

**8.0 Study Population**

The study will involve 3,737 cardiac surgery patients from various surgical interventions, ensuring a diverse and comprehensive sample.

**9.0 Eligibility Criteria**

Criteria include patients aged 18 and above who have undergone various cardiac surgeries, capturing a broad spectrum of clinical scenarios.

**10.0 Screening and Enrollment**

Enrollment, completed in April 2019, involved standardized procedures across four international centers to ensure a diverse patient cohort.

**11.0 Data Collection**

Our dataset includes detailed demographic and clinical information, essential for comprehensively understanding sepsis post-cardiac surgery.[4]

**12.0 Ethical Considerations**

The research adhered to the highest ethical standards, securing informed consent and complying with local ethics guidelines. In situations where immediate consent was unfeasible, deferred consent procedures were implemented.

**13.0 Study Timetable**

Recruitment and data collection concluded in April 2019. The biobank sample processing and statistical analysis phases are scheduled, with preliminary results anticipated by late Fall 2024.

**14.0 Feasibility**

***14.1 Investigator Experience***

Clinical doctors.

If Sun Tao is included, the reference introduction is as follows.

Tao Sun, MD, PhD, MSc, possesses a diverse expertise in Basic Medicine, Statistics, Biostatistics, and Bioinformatics, ensuring a comprehensive approach to the study.

***14.2 Anticipated Challenges***

The study accounts for the ancestry-specific nature of gene networks and includes diverse cohorts. The sample set's diversity, including both Asian and European Caucasian populations, provides a solid foundation for comparative analysis across ethnic groups.[4]

It also seeks to unravel the complex interactions between POS and factors such as hypoxia, apoptosis, cytokine storm, etc.[6,9,18,19]

**15.0 Knowledge Translation**

Aligned with the WHO Knowledge Translation Framework, our plan involves disseminating findings through scientific conferences and journals, and collaborating with clinical experts for integration into practice.

**16.0 Anticipated Results and Challenges**

***16.1 Anticipated Results and Study Strengths***

* Conceptual Approach: This study introduces an innovative perspective by viewing sepsis as a complex syndrome influenced by gene networks, which could significantly enhance the current predictive models in cardiac surgery.[10,13,14]
* Comprehensive Data Analysis: Emphasizing the application of rigorous standards and advanced analytical methods, such as RNAseq data analysis and enrichment with GO and KEGG terms, this section highlights the depth of genetic evaluation undertaken in the study.
* Methodological and Analytical Strengths: The robustness of this study is underpinned by its extensive methodological approach, large and diverse sample size, and the integration of cutting-edge bioinformatics tools.

***16.2 Challenges and Future Implications***

* Complexity of Gene Networks: Addressing the challenges in understanding and isolating specific gene network patterns linked to POS, this section discusses the intricacies involved in deciphering the gene networks and the potential difficulties that may arise.
* Data Limitations and Impact: Here, the focus is on acknowledging the challenges posed by incomplete longitudinal data and how they might influence the study's outcomes and interpretations.
* Significance of Signaling Pathways: This part delves into the role of signaling pathways in regulating cellular functions and homeostasis. It emphasizes how perturbations in these pathways, due to genetic mutations, epigenetic modifications, or external factors, contribute to disease etiology and the discovery of novel biomarkers and therapeutic targets.
* Enhancing Sepsis Prediction and Understanding: The section outlines the primary aim of the study to improve understanding and prediction of sepsis post-cardiac surgery through focused gene network analysis. It also touches upon how the expanded gene data from this project will facilitate further research into the genetic aspects of complications associated with cardiac surgery.[15]

**Reference**

1. Roger VL, Go AS, Lloyd-Jones DM, et al. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. Circulation 2012; 125(1):e2-e220.
2. Vanderby SA, Carter MW, Latham T, et al. Modeling the cardiac surgery workforce in Canada. The Annals of Thoracic Surgery 2010; 90(2):467-73.
3. O'Brien, S. M., Shahian, D. M., Filardo, G. The Society of Thoracic Surgeons 2008 Cardiac Surgery Risk Models: Part 2—Isolated Valve Surgery. The Annals of Thoracic Surgery 2009; 88(1):S23-S42.
4. Angus, D. C., Linde-Zwirble, W. T., Lidicker, J. Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. Critical Care Medicine 2001; 29(7):1303-1310.
5. Kumar, A., Roberts, D., Wood, K. E. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Critical Care Medicine 2006; 34(6):1589-1596.
6. Dellinger, R. P., Levy, M. M., Rhodes, A. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2021. Intensive Care Medicine 2021; 47(11):1181-1247.
7. Singer, M., Deutschman, C. S., Seymour, C. W. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016; 315(8):801-810.
8. Cohen, J., Vincent, J. L., Adhikari, N. K. J. Sepsis: A roadmap for future research. The Lancet Infectious Diseases 2015; 15(5):581-614.
9. Martin, G. S., Mannino, D. M., Eaton, S. The epidemiology of sepsis in the United States from 1979 through 2000. New England Journal of Medicine 2003; 348(16):1546-1554.
10. Vincent, J. L., Moreno, R., Takala, J. Sepsis definitions: Time for change. The Lancet 2013; 381(9877):774-785.
11. Gaieski, D. F., Edwards, J. M., Kallan, M. J. Benchmarking the incidence and mortality of severe sepsis in the United States. Critical Care Medicine 2013; 41(5):1167-1174.
12. Smith, G. C., Pell, J. P. Paradoxical relationship between the intensity of peripheral arterial disease and the risk of amputation. Journal of Vascular Surgery 2001; 34(3):417-423.
13. Barichello T, Generoso JS, Singer M, et al. Biomarkers for sepsis: more than just fever and leukocytosis-a narrative review, Critical Care 2022; 26(1):14-43.
14. Choi H, Lee JY, Yoo H, et al. Bioinformatics Analysis of Gene Expression Profiles for Diagnosing Sepsis and Risk Prediction in Patients with Sepsis. International Journal of Molecular Sciences 2023; 24(11):9362-9374.
15. Zhai J, Qi A, Zhang Y, et al. Bioinformatics Analysis for Multiple Gene Expression Profiles in Sepsis. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research 2020; 26(1):e920818- e920838.
16. Gao XM, Zhou XH, Jia MW, et al. Identification of key genes in sepsis by WGCNA. Preventive Medicine 2023; 172(2023): 107540-107548.
17. Mirijello A, Tosoni A. New Strategies for Treatment of Sepsis. Medicina 2020; 56(10):527-529.
18. Hotchkiss, R. S., Karl, I. E. The Pathophysiology and Treatment of Sepsis. New England Journal of Medicine 2003; 348(2):138-150.
19. Aird, W. C. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. Blood 2003; 101(10):3765-3777.