G: profiler Results:

Functional Enrichment Analysis

To elucidate the biological roles of the 55 sepsis-related genes, a functional enrichment analysis was performed using **g:Profiler**, which integrates multiple functional annotation databases, including Gene Ontology (GO), KEGG pathways, and Reactome. The analysis identified significant Gene Ontology terms, pathways, and cellular components that provide insights into the molecular and cellular mechanisms underlying sepsis. These findings highlight the critical roles of these genes in immune regulation, inflammatory processes, and pathogen recognition, all of which are central to the pathophysiology of sepsis.

ID	Source	Term ID	Term Name	p_{adj} (query_1)
1	GO:MF	GO:0005102	signaling receptor binding	1.008×10^{-22}
2	GO:MF	GO:0001530	lipopolysaccharide binding	3.965×10^{-7}
3	GO:MF	GO:0140375	immune receptor activity	2.166×10^{-6}
4	GO:MF	GO:0019955	cytokine binding	5.379×10^{-5}
5	GO:MF	GO:0001875	lipopolysaccharide immune receptor activity	1.707×10^{-4}
6	GO:BP	GO:0006954	inflammatory response	1.729×10^{-45}
7	GO:BP	GO:0007159	leukocyte cell-cell adhesion	1.154×10^{-25}
8	GO:BP	GO:0060559	positive regulation of calcidiol 1-monooxygena...	3.334×10^{-5}
9	GO:BP	GO:0043388	positive regulation of DNA binding	1.182×10^{-4}
10	GO:BP	GO:0043281	regulation of cysteine-type endopeptidase acti...	2.171×10^{-4}
11	GO:BP	GO:0032963	collagen metabolic process	4.714×10^{-4}
12	GO:BP	GO:0045944	positive regulation of transcription by RNA pol...	7.756×10^{-4}
13	GO:BP	GO:0003158	endothelium development	2.503×10^{-3}
14	GO:BP	GO:0019233	sensory perception of pain	4.517×10^{-3}
15	GO:BP	GO:0061844	antimicrobial humoral immune response media...	4.797×10^{-3}
16	GO:CC	GO:0030141	secretory granule	1.024×10^{-14}
17	GO:CC	GO:0009986	cell surface	1.206×10^{-14}
18	GO:CC	GO:0005576	extracellular region	7.713×10^{-14}

1. Biological Processes (GO:BP)

The analysis revealed a strong enrichment in biological processes associated with immune response and inflammation, which are hallmark features of sepsis. The top terms include:

- **Inflammatory response** (*padj* = 1.79e-45): Sepsis is often triggered by a massive inflammatory response to infection, characterized by the production of pro-inflammatory cytokines, chemokines, and acute-phase reactants. Excessive or

dysregulated inflammation can lead to **tissue damage**, **coagulopathy**, and **multi-organ failure,** the hallmarks of severe sepsis or septic shock.

- **Leukocyte cell-cell adhesion** (*padj* = 1.15e-25): This term highlights the importance of immune cell interactions in mediating cellular migration, adhesion, and communication during the immune response. Leukocyte adhesion is essential for directing immune cells to infection sites. In sepsis, this process can become hyperactivated, resulting in endothelial damage, vascular permeability, and compromised tissue perfusion. Genes involved in cell–cell adhesion often regulate critical steps in immune surveillance and may serve as targets for controlling excessive inflammation.

- **Regulation of Cysteine-Type Endopeptidase Activity** (e.g., Apoptosis/Pyroptosis): Refers to controlling the activity of cysteine proteases (e.g., caspases), which play key roles in programmed cell death (apoptosis) and inflammatory cell death (pyroptosis). Apoptosis of immune cells can weaken host defenses, while pyroptosis can exacerbate inflammation by releasing intracellular contents into tissues. Dysregulated cell death pathways can lead to immune system dysfunction (both hyper and hypoinflammatory states), a major problem in sepsis pathogenesis and progression.

- **Endothelium development** (*padj* = 2.50e-03): Vascular integrity is crucial in sepsis, as endothelial dysfunction often leads to increased vascular permeability and circulatory collapse. Endothelial cells become activated in response to pathogen-associated molecular patterns (e.g., lipopolysaccharide) and proinflammatory signals, upregulating adhesion molecules (ICAM, VCAM) and procoagulant surfaces. This contributes to microthrombi formation, impaired blood flow, and tissue hypoperfusion.

- **Positive regulation of DNA binding** (*padj* = 1.18e-04): This term reflects transcriptional regulatory mechanisms necessary for activating immune-related genes during sepsis progression. Processes that increase the ability of transcription factors (TFs) to bind DNA and modulate gene expression. Sepsis involves extensive transcriptional reprogramming of immune cells, endothelial cells, and other tissues in response to infection and inflammation. TFs like NF-κB, AP-1, and STATs can be

overactivated in sepsis, driving the production of inflammatory mediators. Genes that enhance TF binding may indicate pathways that amplify or sustain inflammatory signals, contributing to cytokine storms.

- **Collagen Metabolic Process (Tissue Remodeling):** Involves the synthesis, modification, or degradation of collagen, the primary structural protein in connective tissues. In chronic or severe inflammation, collagen metabolism may be dysregulated, affecting wound healing, tissue fibrosis, or vascular integrity. Sepsis can lead to tissue damage and multiorgan dysfunction, so changes in collagen metabolism can be a sign of pathological remodeling and impaired recovery. Monitoring genes related to collagen processes might help predict organ damage or long-term sequelae of sepsis.

These enriched processes align with known mechanisms of sepsis, where excessive and dysregulated inflammatory responses contribute to disease severity.

2. Molecular Functions (GO:MF)

The molecular functions enriched in this gene set provide insights into the mechanisms through which these genes mediate immune responses. Key terms include:

- **Signaling receptor binding** (*padj* = 1.00e-22): This term refers to molecules that bind to receptors involved in a wide range of signal transduction pathways—this can include cytokine receptors, growth factor receptors, or pattern recognition receptors. In sepsis, many signaling pathways (e.g., NF-κB, JAK/STAT) become overactivated once receptors sense pathogen-derived signals or stress signals from damaged host cells. Proteins that bind to or modulate these receptors can amplify or dampen the inflammatory response. Thus, genes coding for signaling receptor ligands or binding proteins are crucial in shaping the immune and inflammatory landscape during sepsis.

- **Lipopolysaccharide binding** (*padj* = 3.96e-07): This term is particularly relevant in sepsis caused by Gram-negative bacteria. LPS is a potent endotoxin that triggers a strong innate immune response. When bound by toll-like receptor 4 (TLR4) or other

LPS-binding proteins, it initiates signaling cascades that lead to pro-inflammatory cytokine production. This process is key to bacterial clearance, but excessive or uncontrolled LPS-triggered inflammation can drive septic shock and multiorgan failure.

- **Immune receptor activity** (*padj* = 2.16e-06): Genes involved in this function are integral to the activation of immune signaling pathways, such as cytokine and chemokine signaling, which orchestrate the immune response to infection. Overactivation of immune receptors can lead to massive cytokine release (often referred to as a cytokine storm), contributing to septic shock. Genes encoding immune receptors are often upregulated or altered in septic patients, making them potential diagnostic markers or therapeutic targets.

- **Cytokine Binding:** Proteins with *cytokine binding* activity specifically bind to cytokines—small signaling proteins that regulate immune and inflammatory responses (e.g., interleukins, interferons, tumor necrosis factor). Cytokine storms—an excessive release of pro-inflammatory cytokines—are a hallmark of severe sepsis. Genes encoding cytokine-binding proteins may act as regulators (either increasing or decreasing cytokine effects) and are therefore key in controlling inflammation.

These molecular functions emphasize the critical roles of pathogen recognition and immune receptor signaling in initiating and regulating the host response to sepsis.

3. Cellular Components (GO:CC)

The enrichment of cellular component terms sheds light on the subcellular localization and functional context of the proteins encoded by the genes:

- **Secretory granule** (*padj* = 1.02e-14): Secretory granules store and release inflammatory mediators such as cytokines, which are central to the immune response in sepsis. Immune cells like neutrophils, macrophages, and mast cells degranulate to release enzymes, toxic substances, and signaling molecules to combat pathogens. Excessive or uncontrolled degranulation can contribute to tissue damage and systemic inflammation seen in sepsis. Genes tied to secretory granules may indicate how aggressively immune cells respond, influencing disease severity.

- **Extracellular region** (*padj* = 7.71e-13):Many cytokines, chemokines, and antimicrobial peptides are secreted into the extracellular space, modulating immune cell recruitment and inflammatory cascades. Extracellular proteins can be measured directly in blood samples, making them prime biomarker candidates for early diagnosis and monitoring of sepsis progression.

- **Cell surface** (*padj* = 1.20e-14): Proteins localized to the cell surface, such as receptors and adhesion molecules, are crucial for cellular interactions during immune responses. Receptors on immune cells (e.g., TLRs, cytokine receptors) and adhesion molecules (e.g., ICAM, VCAM) are critical for pathogen recognition, cell migration, and cell-cell interactions in the inflammatory response.Cell-surface molecules are accessible to antibody-based detection (e.g., flow cytometry), facilitating rapid immunophenotyping in suspected sepsis patients.

These enriched components highlight the extracellular and membrane-associated activities of the sepsis-related proteins, consistent with their roles in immune signaling and pathogen recognition.

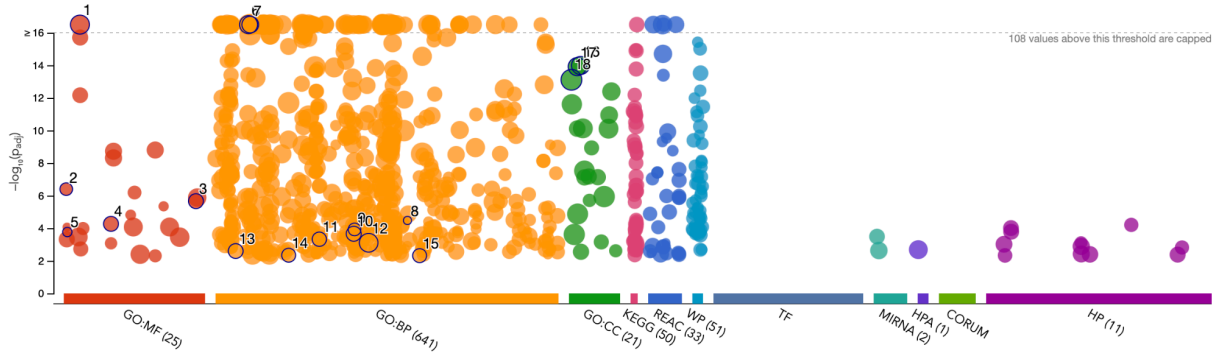
4. Pathophysiological Relevance to Sepsis

The results strongly suggest that the selected genes are deeply involved in key processes that drive sepsis pathogenesis. Terms such as **inflammatory response**, **lipopolysaccharide binding**, and **leukocyte cell-cell adhesion** are directly related to immune dysregulation, endothelial dysfunction, and systemic inflammation—hallmarks of sepsis. These findings underscore the biological relevance of these genes as potential biomarkers or therapeutic targets.

Additionally, terms associated with transcriptional regulation and protein secretion reflect the broader cellular responses to infection, including cytokine release and immune cell activation. For example, the enrichment of **endothelium development** provides insights into vascular changes that contribute to sepsis-induced organ damage.

5. Visualization of Results

The functional enrichment results are visualized in a scatterplot, where each dot represents an enriched term. The terms are grouped by categories such as **GO:MF**, **GO:BP**, and **GO:CC**, and their significance is represented by the y-axis (`-log10(p-value)`). The most significant terms, such as **inflammatory response** and **signaling receptor binding**, are highlighted with the highest y-axis values, reflecting their strong statistical significance.



6. Implications for Diagnostic Gene Discovery

The functional enrichment analysis provides a strong foundation for further exploration of these genes as sepsis biomarkers. Genes associated with highly enriched terms, such as **lipopolysaccharide binding** or **inflammatory response**, are particularly promising for diagnostic applications. Their involvement in pathogen recognition and immune regulation suggests that these genes could help distinguish sepsis from other inflammatory conditions, potentially enabling earlier and more precise diagnoses.