Identification of Potential Prognosticators for Sepsis through Expression Analysis of Transcriptomic Data from Sepsis Survivors and Nonsurvivors

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ABSTRACT

Background. Infection can be severely complicated by a dysregulated, whole-body inflammatory response known as sepsis. While previous research showed that genetic predisposition is linked to outcome differences, current patient characterization fails to determine which septic patients have greater tendencies to develop into severe sepsis or go into septic shock. As such, the identification of prognostic biomarkers may assist in identifying these high-risk patients and help improve the clinical management of the disease.

Objective. In this study, we aimed to identify molecular patterns involved in sepsis. We also aimed to identify essential genes associated with the disease's survival which could serve as potential prognosticators for the disease.

Methods. We used weighted gene co-expression analysis (WGCNA) to analyze GSE63042, an RNA expression dataset from 129 patients with systemic inflammatory response syndrome or sepsis, including 78 sepsis survivors and 28 sepsis nonsurvivors. This analysis included identifying gene modules that differentiate sepsis survivors from nonsurvivors and qualitatively assessing differentially expressed genes. We then used STRING's protein-protein interaction and gene ontology analysis to determine the functional and pathway relationships of the genes in the top modules. Lastly, we assessed the prognosticator abilities of the hub genes using ROC analysis.

Results. We found four diverse co-expression gene modules significantly associated with sepsis survival. Our differential gene expression analysis, combined with protein-protein interaction and gene ontology analysis, revealed that the hub genes of these modules – *TAF10*, *SNAPIN*, *PSME2*, *PSMB9*, *JUNB*, and *CEBPD* – may serve as candidate markers for sepsis prognosis. These markers were significantly downregulated in sepsis nonsurvivors compared with sepsis survivors.

Conclusion. Weighted gene co-expression analysis, gene ontology enrichment analysis, and protein-protein network interaction analysis of transcriptomic data from sepsis survivors and nonsurvivors revealed *TAF10*, *SNAPIN*, *PSME2*, *PSMB9*, *JUNB*, and *CEBPD* as potential biomarkers for sepsis prognosis. These genes are associated with functions related to proper immune response, and their downregulation in sepsis nonsurvivors suggests eventual immune exhaustion in late sepsis. Further analyses, however, are necessary to validate their roles in sepsis progression and patient survival.

Keywords: sepsis survival, prognosis, TAF10, SNAPIN, PSME2, PSMB9, CEBPD, JUNB

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INTRODUCTION

Sepsis is a life-threatening condition characterized by organ dysfunction resulting from a dysregulated host response to infection.[1] This condition can progress into septic shock, a subset of sepsis with circulatory and cellular or metabolic dysfunction associated with increased mortality risk.[2] Due to its high incidence and mortality rates, sepsis is a major public health concern.[3,4] According to the World Health Organization, the number of sepsis cases reached more than 40 million in 2017. In the same year, sepsis has affected 11 million deaths globally, accounting for nearly 20% of deaths worldwide.[5] The global incidence of septic shock was 22 to 240 cases for every 100,000 sepsis patients.[6] In the Philippines, the case fatality rate reached 30% for sepsis and up to 80% for septic shock among hospitalized patients and is considered one of the primary causes of patient mortality.[7,8]

In sepsis prevention and detection, using prognosticators to identify patients genetically predisposed to septic shock may significantly improve the condition's mortality rate. With accurate molecular prognosticators, it is possible to predict the survival of patients diagnosed with sepsis. This tool can be an invaluable adjunct to the current clinical and pathological parameters in identifying patients with higher mortality risk.[9]

Biomarkers associated with the magnitude of the inflammatory response, like *IL-6* and *CXCL10*, were correlated with sepsis patients' clinical outcomes in population-based studies.[10,11] However, such biomarkers' ability to predict outcome in an individual is limited, primarily due to the lack of specificity of the biomarkers and their similarity to the early inflammatory response.[2] Moreover, such markers were observed using a parallel comparison between afflicted and non-afflicted, making it impossible to assess their prognostic value. Thus, there is a need to identify more specific and unique biomarkers to serve as reliable prognosticators for sepsis. Furthermore, a local investigation of candidate biomarkers may provide insight into the differences in the types of molecules associated with sepsis between populations. The identified biomarkers may be helpful for clinical application in the local populations.

In this study, an mRNA expression dataset containing transcriptional data derived from 129 patients derived from a longitudinal study was analyzed for genes that may differentiate sepsis patient survival and serve as prognosticators. Here, we present six candidate genes and elaborate on their relevance to immune response and sepsis survival.

METHODS

DataSet

The Gene Expression Omnibus (GEO) DataSets (http://www.ncbi.nlm.nih.gov/geo/gds) of the National Center of Biotechnology Information (NCBI) was our database of choice for dataset searching. As shown in Figure 1, we used the term "((sepsis) AND ((survival) OR prognosticator))" as the search query, and organism: "homo sapiens" and entry type: "DataSets" or "Series" as additional search filters. We then screened the resulting datasets based on suitability to the study objectives and sample size. Since we aimed to uncover potential prognosticators for sepsis survival, a dataset was deemed suitable if it contained the recommended number of samples (larger than 20) for both sepsis survivor and nonsurvivor groups.[11]

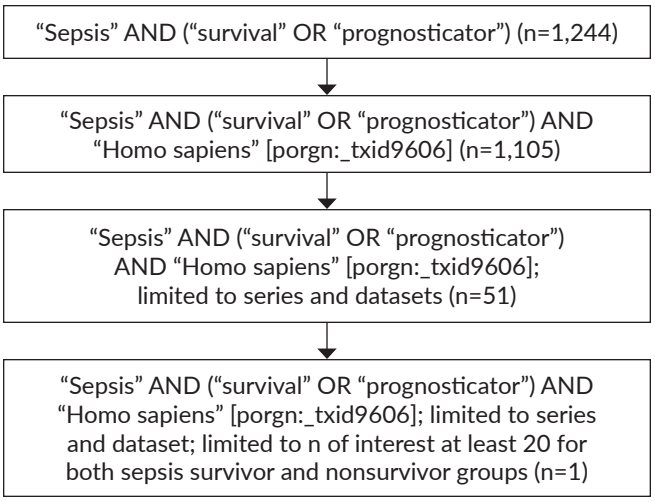

Figure 1. The search algorithm for the study dataset.

Ultimately, we used GSE63042 as our DataSet. GSE63042 contained 129 transcriptional data from peripheral blood of patients with systemic inflammatory response syndrome (SIRS) or sepsis (infection with SIRS), indicating that 78 were sepsis survivors and 28 were sepsis nonsurvivors. There were also identifiers of disease severity, with 21 sepsis patients classified as severe sepsis and 33 survivors with septic shock experience.

WGCNA Network Construction and Module Identification

Weighted Gene Network and Co-Expression Network Analysis (WGCNA) of R software (version 4.0.2) was used to perform gene expression analysis. First, sample cluster analysis was performed where obvious outliers were detected and eliminated. After outlier exclusion, re-clustering was done according to gene expression levels in each sample to uncover the correlation between samples.

For gene module detection, a soft-thresholding power of 12 was used according to the rule of the scale-free network, and the minimum power value at the plateau was taken as the parameter of the subsequent analysis. A gene clustering tree was constructed based on the correlation of intergene expression levels. Afterwards, gene modules were detected using the dynamic tree cut function with the minimum number of genes in the module set to 30. Modules with similar expression patterns were merged according to the

similarity of the module eigenvalues. A sample expression pattern heat map was then drawn.

Dissimilarity of the topological overlap measure (TOM) was used to determine the degree of correlation between genes. Statistical significance among the modules and clinical traits was verified using the Pearson correlation test, and a $p<0.05$ was considered statistically significant. For this study, "sepsis alive vs. dead" was analyzed as the clinical trait of interest. The top four modules with the highest weighted correlation coefficient scores among all modules were chosen as modules of interest for further analysis. Gene significance and module membership were also calculated to relate the modules to the clinical trait.

Network and Enrichment Analysis

To determine associated functional ontogenies of the differentially expressed genes, network prediction and enrichment analysis were made using STRING (https://string-db.org/). "Multiple protein" option search was used to search for corresponding protein networks. Simultaneously, proteins within each module were inputted in the "List of Names" box with one protein per line. The specific organism for the query was set to humans (*Homo sapiens*). The analysis was done for the four top modules. The results were generated using medium confidence (0.400) setting for the minimum required interaction score with a maximum number of 10 for 1st shell node interactions.

Other Data Analytics

Continuous clinical data and gene expression levels to compare interest groups were analyzed using student t-tests. Categorical clinical data were compared using chi-square tests comparing two proportions. Mean with standard deviation were indicated, with differences determined at $p<0.05$. Receiver operator curves (ROC) were computed with a determination of areas under the curve (AUC) for selected candidate genes for prognostication.

Validation of Findings in an Independent Dataset

To evaluate the prognostic value of each candidate gene in an external dataset, we performed ROC curve analysis on an independent GEO dataset. GEO was searched, focusing on the same query as that of the primary dataset, which includes "((sepsis) AND ((survival) OR prognosticator))" as the search query, and organism: "homo sapiens" and entry type: "DataSets" or "Series" as additional search filters. The inclusion criteria for the validation dataset were as follows: expression data is from peripheral blood samples of sepsis patients, includes gene expression profiles for all identified hub genes from the primary dataset, and has a sample size of ≥10 for each group. Based on these criteria, we identified GSE95233 as the appropriate validating dataset. GSE95233 includes gene expression and survival data from 51 patients with septic shock - 34 survivors and 17 nonsurvivors.[12]

Ethical Considerations

The UP Manila Ethics Board (UPMREB) classified this study protocol as exempted from ethics review with code 2021-488-EX.

RESULTS

Data set GSE63042, together with its clinical data, was downloaded from the GEO database from the NCBI website. The dataset includes 129 transcriptional data from peripheral blood of patients with systemic inflammatory response syndrome (SIRS) or sepsis (infection with SIRS), indicating that 78 were sepsis survivors and 28 were sepsis nonsurvivors. Aside from survival, the dataset also presented severe sepsis and shock data. However, these

Table 1. Demographic and clinical features of the study population

Clinical variable	SIRS (n=28)	Sepsis survivors (n=78)	Sepsis Nonsurvivors (n=28)	p value*
***Age** (years)*	64.9 ± 14.4	56.1 ± 18.0	67.6 ± 17.0	0.0040
***Gender** (% Male)*	7 (34.80%)	59.00%	60.70%	0.8747
Pathogen				
S. aureus	N/A	26%	18%	0.3631
S. pneumoniae	N/A	26%	14%	0.1446
Enterobacteriaceae	N/A	29%	11%	0.0216
Smoker	21.70%	30.80%	25.00%	0.5503
Alcohol abuse	17.40%	17.90%	10.70%	0.3325
Immunosuppression	0%	6.40%	7.10%	0.9003
Comorbidities				
Neoplastic disease	13.00%	6.40%	21.40%	0.0684
Diabetes	30.40%	32.10%	35.70%	0.7313
Congestive heart failure	0%	6.40%	14.30%	0.2707
Chronic kidney disease	26.10%	21.80%	25.00%	0.7342
Chronic liver disease	8.70%	5.10%	21.40%	0.0453

**p value significance set at <0.05; compared only survivors with nonsurvivors*

data are not longitudinal and may not serve to predict patients. Nevertheless, we included comparative statistics to contextualize some realistic perspectives.

Patient demographics and available clinical data are summarized in Table 1. A comparison of demographics of the survivors (n=78) and nonsurvivors (n=28) reflected the expected clinical profile for more severe diseases. Enterobacteriaceae infection is higher among nonsurvivors. The nonsurvivors were generally older. Liver disease is also significantly higher in nonsurvivors. These reflect that several factors may influence mortality in sepsis. Nonetheless, a common host response or deficiency in these protean conditions may explain the susceptibility of individuals to more severe conditions.

Initial sample cluster analysis measuring signal intensities revealed three sample outliers. Three samples (designated as SEPSHK31, SEPUNC13, and SEPUNC14) were excluded based on the resulting sample clustering, shown in Figure 2. The resulting expression matrix containing 10,000 genes from 126 patient samples was used for the WGCNA analysis.

Sample re-clustering according to gene expression levels in each sample resulted in a cluster tree depicting the relationship among the samples. There was no bias regarding signals' differences and overall averages with survival.

For WGCNA, we screened for the soft-threshold power using a scale-free topology index fit (Figure 3). Based on the results, we chose the power value 12, where scale independence was above 0.8 and mean connectivity had a relatively high value. With the set soft power and minimum module size defaultly set at 30, WGCNA module identification resulted in forty distinct gene co-expression modules arbitrarily designated with unique module colors. We then computed module eigengene values and clustered the modules based on the calculated values (Figure 4). Finally, we combined closely related sets by merging modules close to each other using a cutoff of 0.2. This merging resulted in 15 modules named using unique colors. Figure 5 shows the cluster dendrogram of the modules.

We then quantified the correlations between the co-expression modules and the measured clinical traits. The correlation between clinical traits and merged gene modules is shown in Figure 6. From this heatmap, co-expression modules that are significantly associated with various clinical traits can be identified. For survival as outcome, four modules are significant, namely, MEplum1 (correlation index: -0.32), MEsteelblue (correlation index: -0.3), MEbrown (correlation index: -0.29), and MEgrey60 (correlation index: -0.23). Interestingly, all four were negatively correlated with sepsis survival, with general downregulation in nonsurvivors. In addition to survival, the present data also showed parallel comparisons among various severity states. A composite of severe, shock, and non-surviving patients in complicated states were found to have downregulated MEbrown modules. It is surprising, however, that aside from this module, our results showed minimal differences in the transcriptional profiles of the various severity, especially among survivors.

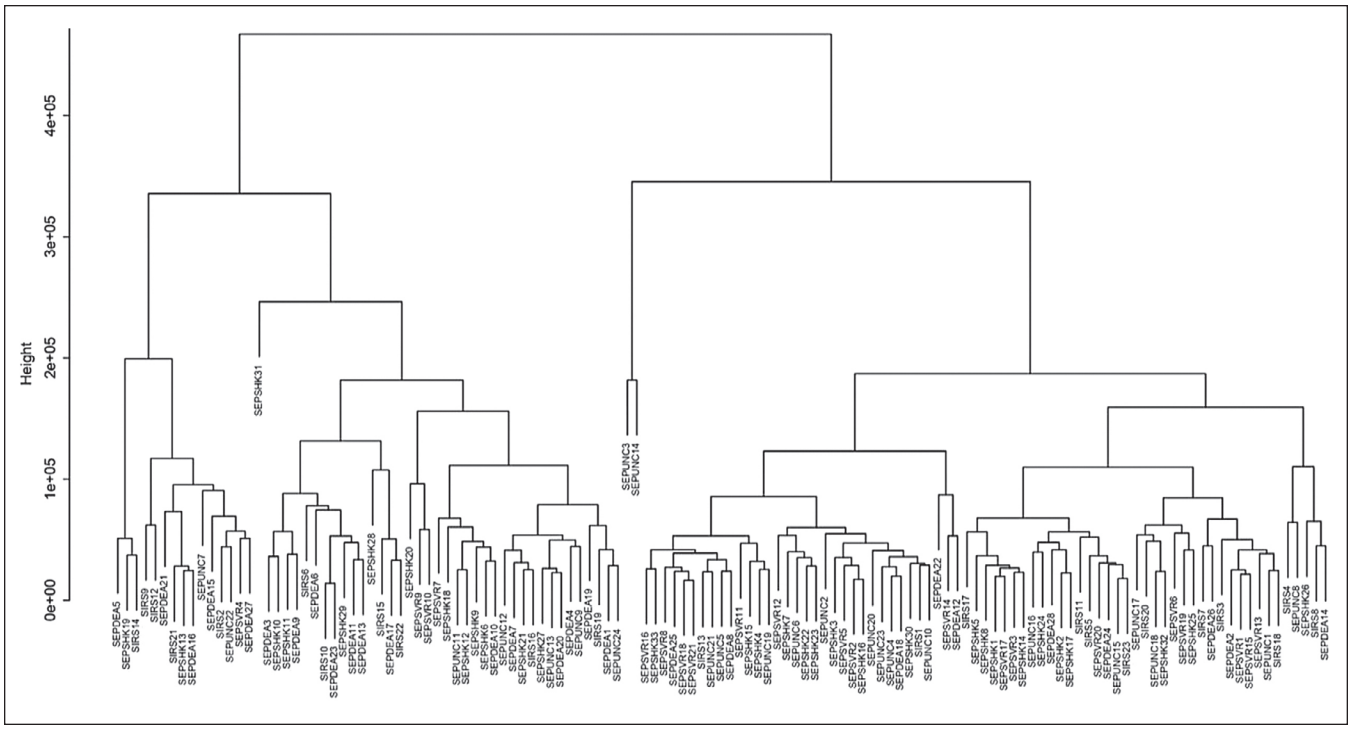

Figure 2. Dendrogram and trait heatmap of study samples showing the insignificant bias of the sample clustering to selected clinical traits. Notably, there was no bias in the clustering pertinent to survival.

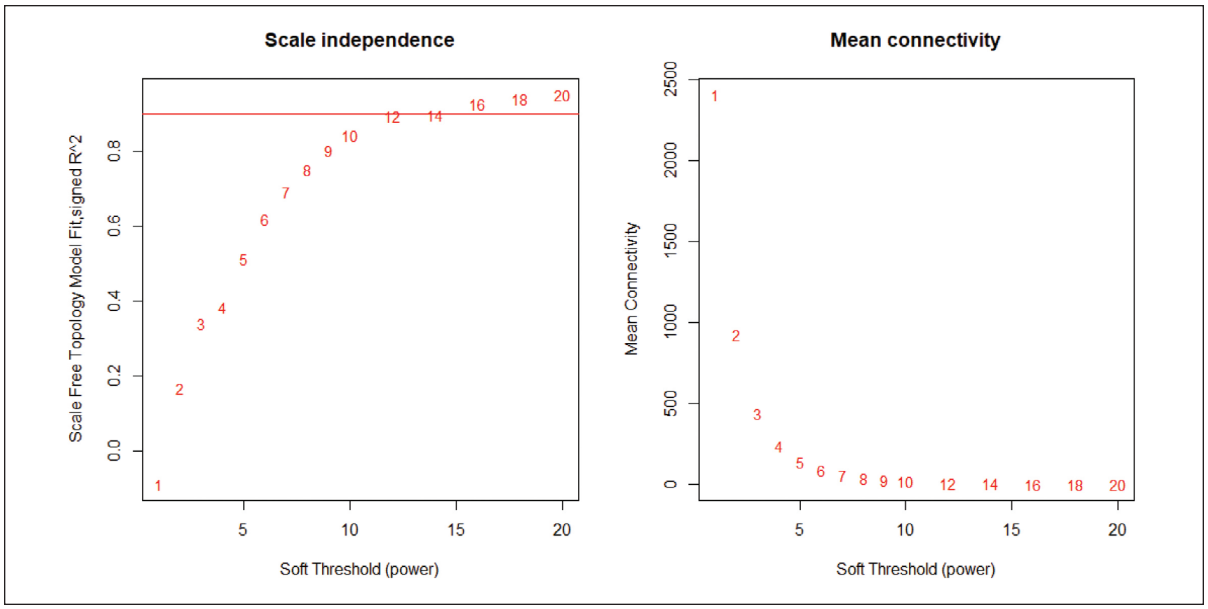

Figure 3. Scale independence and mean connectivity of different soft-thresholding values. A soft-threshold power was set at 12 based on a scale-free topology fit index with the corresponding mean connectivity near minimum, indicating the discrimination of more influential genes under these conditions.

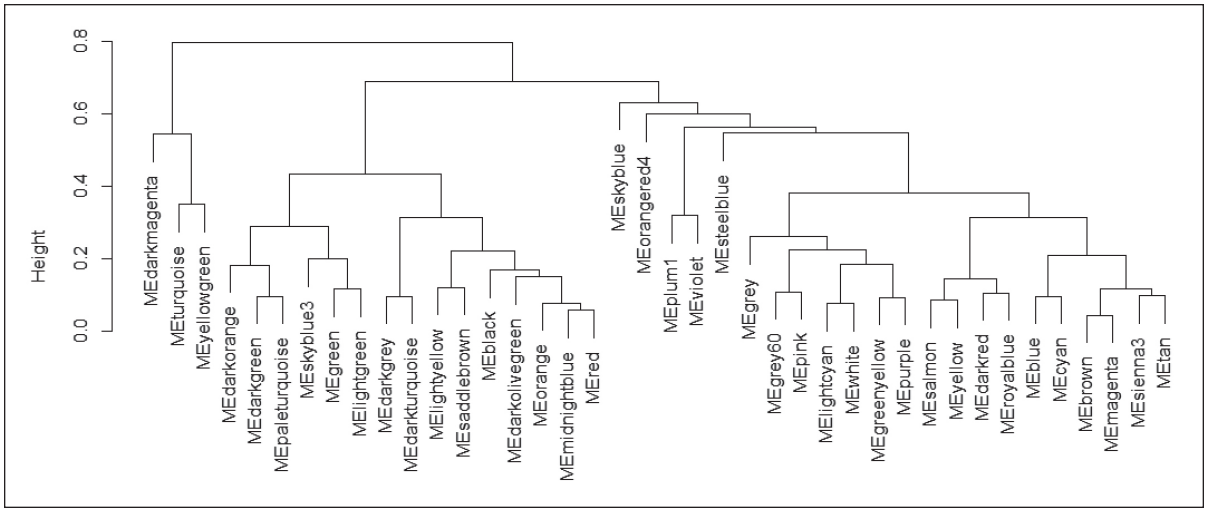

Figure 4. Clustering of module eigengenes. The inferred gene sets were assigned arbitrary colors, and the minimum membership set was at 30 (combining sets is less than 30 with the nearest neighbor).

We then performed GO enrichment analysis for the genes in the four significant co-expression modules. Table 2 shows the resulting top enrichments for the modules, while Figure 7 shows the protein-protein interaction network.

Using $\log_{10}$-fold change ≥2 and $p<0.05$ as cutoff values, differential gene expression (DGE) analysis revealed 1,013 differentially expressed genes, 989 of which were significantly downregulated in sepsis nonsurvivors. Figure 8 shows a volcano plot of the $\log_{10}$FC vs. the p value (expressed as $-\log_{10}$ p value) for all probe sets.

The highly correlated genes of MEsteelblue and MEgrey60 four genes had highly negative $\log_{10}$FC and high degrees of module membership in the immunity-related modules. *PSME2* (proteasome activator subunit 2) and *PSMB9* (proteasome 20s subunit beta 9) are members of MEsteelblue, while *TAF10* (TATA-box binding protein associated factor 10) and *SNAPIN* (SNAP associated protein) are from MEgrey60. These four genes were significantly downregulated in septic patients who died compared with those who survived. In addition, the hub genes of the MEplum1 module, *JUNB,* and *CEBPD* were also signifi-

cantly downregulated in sepsis nonsurvivors. Figure 9 shows the expression of these genes in septic patients.

Next, we determined the prognostic marker potential of the selected genes. In Figure 9, ROC analyses showed a moderately high correlation to survival (AUC: 70-80%).

Lastly, we plotted the ROC curves of the candidate genes using an independent GEO DataSet by Pachot et al. (GSE95233) to assess and validate the prognostic value of the hub genes in an external cohort (Figure 10). Interestingly, the position of the *JUNB* gene (AUC>0.80) as a potential sepsis survival prognosticator is further supported.

DISCUSSION

Tsalik and colleagues' study identified genes encoding for expressed sequence variants that may be causally related to multiple sepsis outcomes.[13] Using the same dataset, our present study uncovered additional molecular patterns involved in sepsis and focused on identifying essential genes associated with the disease's survival. To achieve this, we performed WGCNA, an analysis that can cluster highly correlated genes and identify key module eigengenes or hub genes through network analysis.[14] Our analysis revealed

Table 2. GO enrichment analysis in terms of biological processing of genes in MEsteelblue, MEgrey60, MEbrown, and MEplum1

Module	Term ID	Term Name	*P*-value
MEsteelblue	GO:1900245	Positive regulation of the MDA-5 signaling pathway	0.04910
	GO:0035455	Response to interferon-alpha	2.59×10^{-5}
	GO:0034340	Response to type I interferon	6.72×10^{-12}
	GO:0060337	Type I interferon signaling pathway	1.46×10^{-10}
MEgrey60	GO:0002444	Myeloid leukocyte-mediated immunity	1.45×10^{-15}
	GO:0043299	Leukocyte degranulation	4.02×10^{-15}
	GO:0002446	Neutrophil mediated immunity	7.83×10^{-15}
	GO:0042590	Antigen processing and presentation of exogenous peptide	0.04390
MEbrown	GO:0009058	Biosynthetic process	0.02270
	GO:0044238	Primary metabolic process	0.00033
	GO:0071704	Organic substance metabolic process	0.00033
	GO:0044237	Cellular metabolic process	0.00047
MEplum1	GO:0051254	Positive regulation of RNA metabolic process	0.03850
	GO:0010628	Positive regulation of gene expression	0.05000
	GO:0051173	Positive regulation of nitrogen compound metabolic process	0.05000

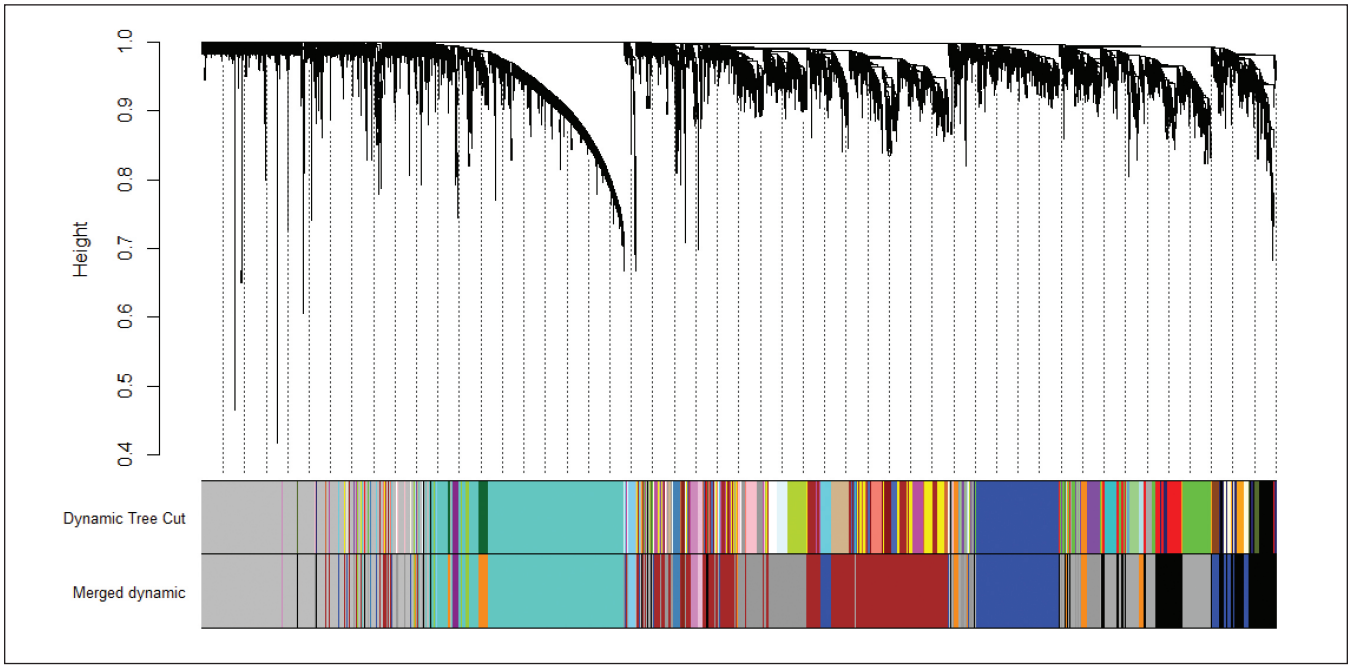

Figure 5. Gene dendrogram of sepsis samples and derived modules. The distinct groups of correlated gene sets are evident by the obvious dips in the branches that are disparate from each other.

Figure 6. Module-trait relationships between clinical traits and module eigengenes. Each cell contains the corresponding correlation coefficients (upper value) and p value (lower value). Four top modules significantly associated with sepsis survival can be identified by focusing on the sepsis-alive-vs-dead column. These include MEplum1 (correlation index: -0.32, p= 0.0002), MEsteelblue (correlation index: -0.3, p= 0.0005), MEbrown (correlation index: -0.29, p= 0.0007), and MEgrey60 (correlation index: -0.23, p= 0.007). These modules are negatively correlated with sepsis survival; their expression levels were significantly lower in patients who died than in those who survived.

co-expression modules associated with the different stages of sepsis. Minimal differences were observed in the various sepsis severity states — SIRS, severe, and shock — ($p>0.05$). However, there were modules correlated with survival (Alive vs. Dead, $p<0.05$). The functional enrichment analysis of the modules using STRING revealed similar functions of some associated modules.

The significance of associated immunity-related modules (MEgrey60 and MEsteelblue) is expected but exciting. The MEsteelblue module was hugely enriched in pathways related to biological processes of defense response to a virus, innate immune response, type I interferon signaling pathway, and defense response to other organisms. Since sepsis pathogenesis is dependent on the innate immune response, perturbations in various innate immunity components can cause the clinical progression of the condition.[15] There is a pronounced innate immunity dysfunction found in endothelial cells, neutrophils, macrophages, monocytes, natural killer (NK) cells, and dendritic cells during sepsis.[16] This condition undermines the host response in eliminating and clearing invading pathogens, including bacteria and viruses. Although the diagnosis of viral sepsis is rare, any virus can induce sepsis in susceptible populations such as neonates and infants.[17] Type I interferons (IFNs) are cytokines secreted by innate immune cells to modulate antiviral and antibacterial immunity and activate the subsequent adaptive immune response.[18,19] However, more research is still required to elucidate the causality between prolonged or halted IFN expression and viral persistence.[20]

MEgrey60 was associated with myeloid leukocyte mediated immunity, leukocyte degranulation, and myeloid cell activation in the immune response. Myeloid-derived suppressor cells are essential in effectively regenerating functional neutrophils, monocytes, and DCs.[16] However,

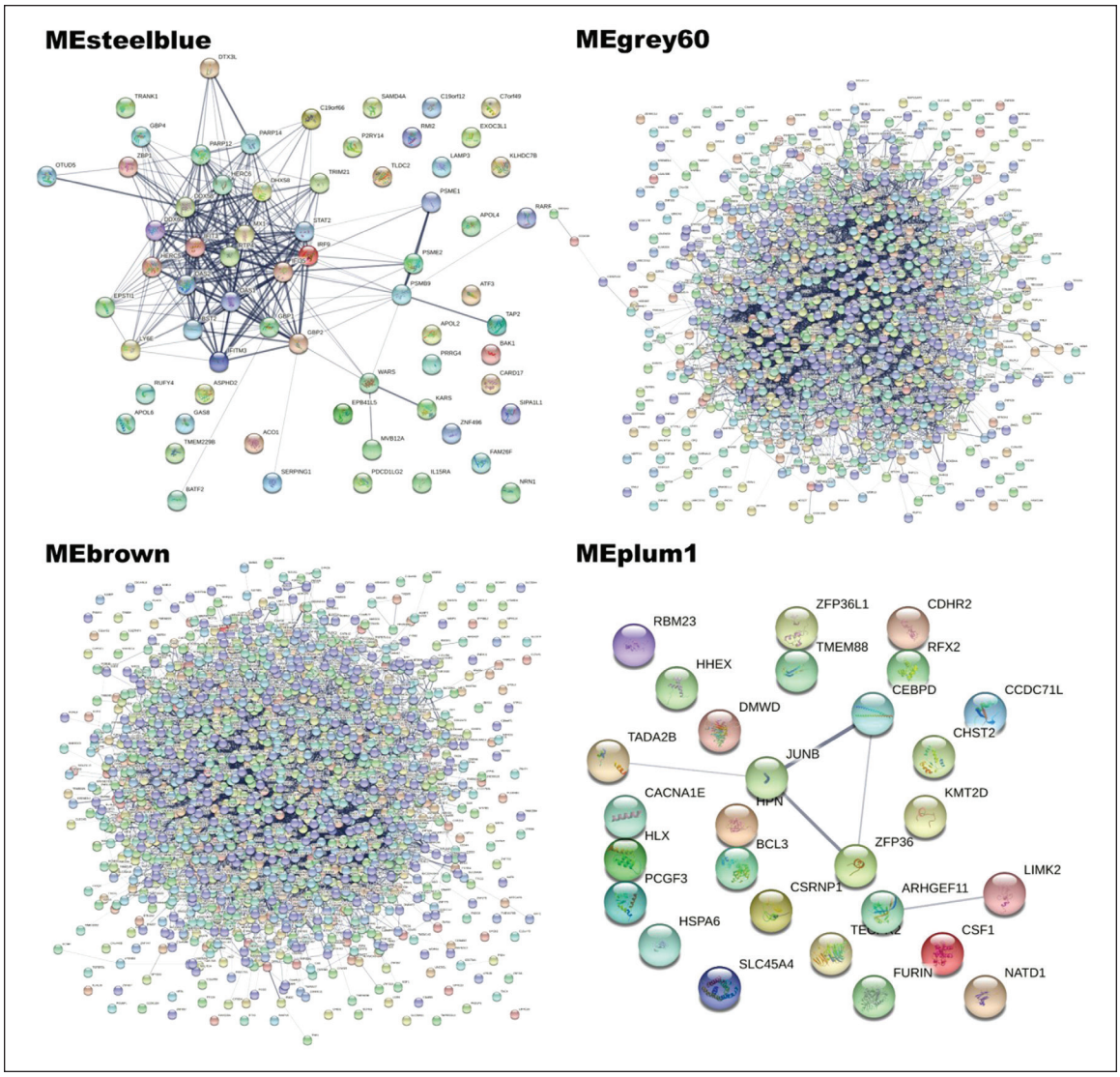

Figure 7. Protein-protein interaction network of modules associated with sepsis survival. A discussion of the inferences is presented in the text.

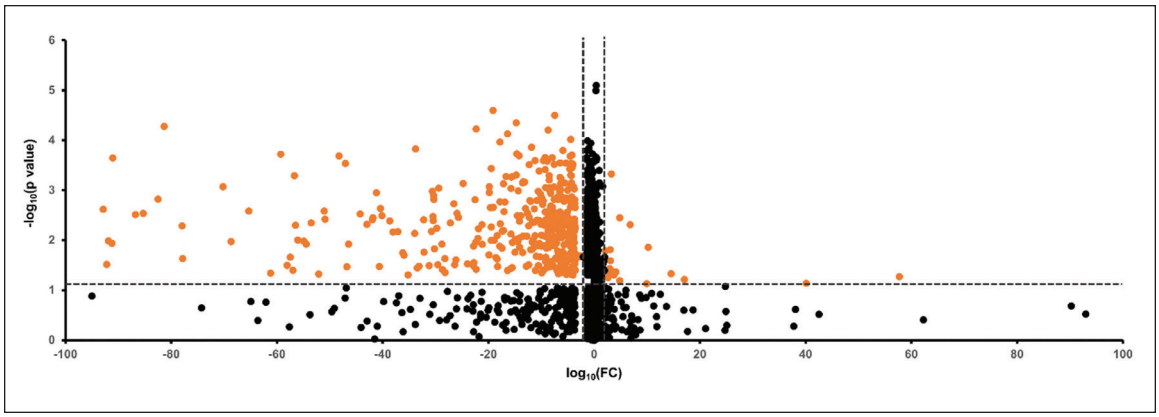

Figure 8. Volcano plot indicating the upregulated and downregulated genes in sepsis nonsurvivors. Differentially expressed genes are highlighted in orange ($p<0.05$). These broadly demonstrate differentially expressed genes expressed less in nonsurvivors (bias to upper left quadrant).

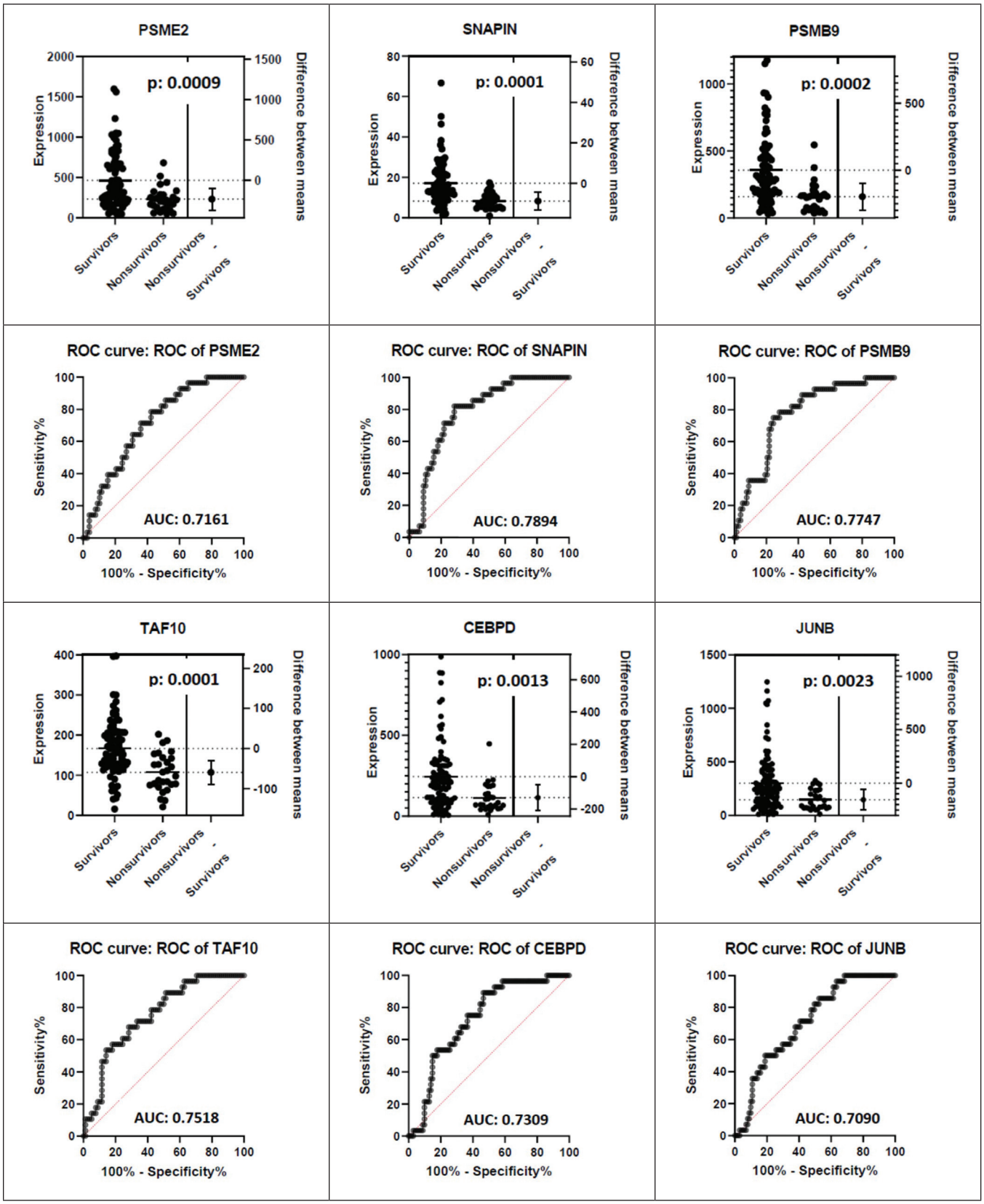

Figure 9. Expression levels of *PSME2*, *PSMB9*, *TAF10*, *SNAPIN*, *CEBPD*, and *JUNB* in sepsis survivors and nonsurvivors ($p<0.05$). All genes had decreased expression in the nonsurvivors. Receiver-operator curve analyses showed moderate to good (70-80%) AUC for all markers.

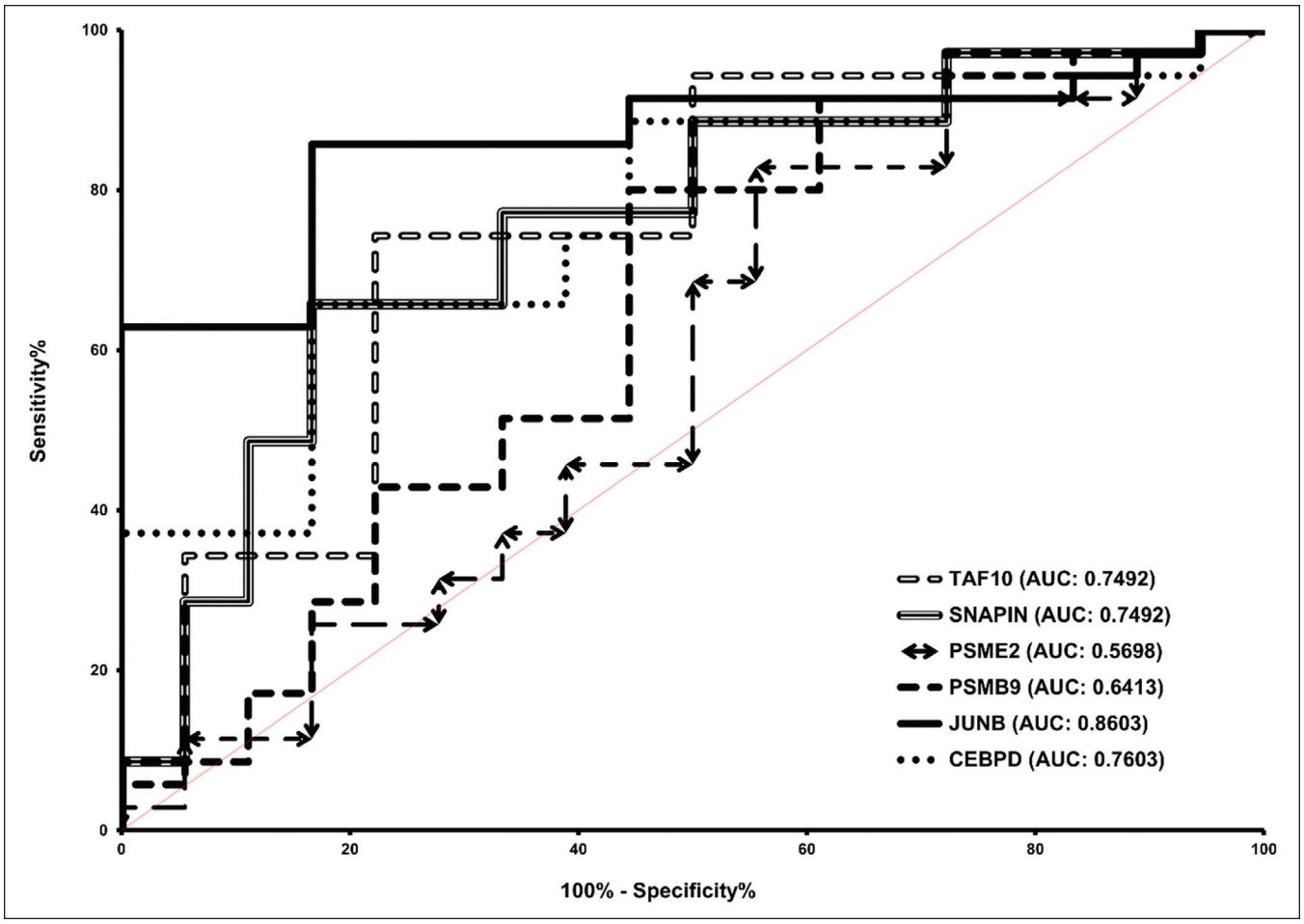

Figure 10. ROC curves of the hub genes *TAF10*, *SNAPIN*, *PSME2*, *PSMB9*, *JUNB*, and *CEBPD* using GSE95233. In this verification dataset, the candidate *JUNB* was found to have an AOC >0.85, making this the most promising prognostication marker for survival among the candidates.

the progressive shift towards immature myeloid cell release over the mature neutrophil release and other myeloid cell dysfunctions during sepsis may orchestrate sepsis-acquired immunodeficiency.[21] Neutrophils may eliminate pathogens through degranulation; however, a population of this cell, known as low-density neutrophils (LDNs), were observed to play a pivotal role in sepsis-induced immune suppression.[22] As possibly depicted by the general downregulation of the modules and genes, these cells are also crucial in immune paralysis – the leading cause of death in most sepsis patients – which occurs after systemic inflammation despite pathogen clearance.[22] Intriguingly, this trend of underactivation is evident in both immune-related modules, MEgrey60 and MEsteelblue. Taken together, these data hint that survival-associated genes may involve multiple innate immune responses towards various pathogen types, and exhaustion of these components encompasses immune paralysis related to patient survival.

DGE analysis, combined with the PPI network interaction analysis, identified the crucial hub genes in these modules. The MEsteelblue module contained the two essential genes: *PSME2* and *PSMB9*. *PSME2* (proteasome activator subunit 2) is a subunit of the 26S proteasome for antigen processing.[23] In a separate gene expression analysis, *PSME2* and other immune response genes had consistently low levels of expression in the melioidosis cohort. This trend suggests an altered defense response or a reduced proteasomal activity at a later infection stage.[24] *PSMB9* (proteasome 20S subunit beta 9) is another human proteasomal subunit found in human monocytes.[25] Together with other immunoproteasome subunits, *PSMB9* was significantly expressed in monocytes in an idiopathic inflammatory myopathies (IIM) study.[26] It was also significantly expressed during the early stages of sepsis.[27] Since our findings revealed its downregulation in patients who ultimately died of sepsis, it may be possible that *PSMB9* was initially expressed in the earlier stages of infection and then declined at the latter stages where immune exhaustion was evident. Notably, a rapid, LAMP-based, 29-mRNA panel for acute infection and sepsis diagnostic and prognostic test included *PSMB9* and is preparing for a

prospective study as part of a registrational trial.[28] Incidentally, both *PSME2* and *PSMB9* genes are induced by gamma-interferon, a type II interferon secreted predominantly by NK cells during an antimicrobial innate immune response.[29-31]

In the grey60 module, *TAF10* and *SNAPIN* genes were amongst the relevant hub genes. *TAF10* encodes for transcription factor IID (TFIID) subunits that coordinate transcription initiation by RNA polymerase II to various activators and repressors.[32] Langley and Kingsmore observed that *TAF10* and other TATA box-binding proteins had reduced expression in sepsis death based on a separate sepsis prognosis biomarker study.[33] However, the exact role of *TAF10* in sepsis needs further elucidation. Meanwhile, *SNAPIN* associates with the SNARE (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor) complex and the BLOC-1 (biogenesis of lysosome-related organelles) complex.[34] *SNAPIN* binds with TLR-2 to activate macrophages, and its expression was deemed nonspecific to diseases.[35] Further studies showed that *SNAPIN* is essential in autophagosomal acidification and maturation. These processes occur by preventing proton leakage in macrophages.[36] Notably, mutations in *SNAPIN* and other BLOC-1 subunits could bring forms of Hermansky-Pudlak syndrome (HPS) characterized by immunodeficiency in some individuals.[37,38] In conjunction with the theme of immune exhaustion in late sepsis, the downregulation of *SNAPIN* expression in sepsis nonsurvivors suggests macrophage dysfunction.

The protein-protein interaction network analysis and GO analysis highlighted only the role of the MEplum1 in transcription regulation. Upon hub gene exploration, however, we found it interesting that two of its genes - *CEBPD* and *JUNB* - also have functions in the immune response.

CEBPD (CCAAT enhancer binding protein delta) is a protein-encoding gene critical for gene regulation in the immune and inflammatory response and possibly with macrophage activation and differentiation.[39] Banerjee et al. reported that *CEBPD* displayed a protective role against underlying sepsis-induced lethality using irradiated *CEBPD*-deficient mice. Their models exhibited increased pro-inflammatory cytokines, including *IL-6 and TNF-α,* and increased expression of chemokines, specifically, *CXCV11, MCP-1, and MIF-1α*.[40] Its ablation in mice models also conferred some protection against pneumococcal pneumonia.[41] However, *Klebsiella pneumoniae*-induced pneumonia did not show a similar trend, where they suspected *CEBPD* affecting macrophage mobilization.[42] Looking at gene expression profiles, Godini et al. reported the upregulation of *CEBPD* in dendritic cells, neutrophils, and macrophages exposed to septic plasma.[43] They verified this in another transcriptome data from children with septic shock.[44] However, further data on *CEBPD* in deceased septic patients are limited.

JUNB (JunB proto-oncogene or AP-1 transcription factor subunit) encodes transcription factors for regulating gene activity after primary growth factor response.[45] The protein product of this gene is involved in various cellular processes, including proliferation and differentiation.[46] Available evidence also showed that this protein has a role in myeloid immune activation. Fontana et al. used macrophages derived from *JUNB*-deficient mice to show that JUNB augments gene expression for cytokines, chemokines, interferon-associated genes, and other immune function genes.[47] Khoyratty et al. also observed a similar trend with neutrophils from *JUNB* knockout mice. They saw that knocking out *JUNB* results in an attenuated *Cav 1* expression, a gene involved in neutrophil activation, adhesion, and transendothelial migration.[48] In addition, this protein orchestrates Treg-mediated immune homeostasis by facilitating IRF4-dependent transcription for effector Treg differentiation.[49,50] In terms of sepsis prognostication, multiple studies have already reported differential gene expression of *JUNB*. Cai et al. included *JUNB* in the differential gene set of monocytes in their research, where they established a diagnostic tool for early sepsis.[51] Another study also reported higher expression of *JUNB* in a sepsis group versus the healthy control.[52] However, our results using two independent datasets showed downregulation of *JUNB* in sepsis nonsurvivors. Since *JUNB* is involved in immune cell activation and proliferation, this downregulation in nonsurvivors could contribute to immune cell exhaustion, particularly in late sepsis.

Altogether, our data support the theme of immune exhaustion in late sepsis. Looking back at Figure 6, we can see that the immunity-related modules (MEsteelblue, MEgrey60, and MEplum1) were downregulated in deceased septic patients but are upregulated in sepsis survivors compared to healthy controls. Consistent with other DEG studies, the hub genes of these modules were mainly upregulated in septic patients compared with healthy controls.[27,51,52] However, disease progression may lead to overwhelming, rampant immune dysfunction and eventual immune exhaustion.

We also noted significant downregulation of the genes in the MEbrown module. This large module involved a wide array of primary metabolic processes and seemed to represent a wide array of metabolic processes affected during the worsening of sepsis.

Looking forward, molecular experiments are required to analyze and verify the role of these genes in sepsis progression and whether these are valuable in prognostications and as sepsis treatment targets.

CONCLUSIONS

In conclusion, our study provided evidence of the diverse co-expression gene modules associated with sepsis survival. Additionally, we identified six key genes, namely, *TAF10, SNAPIN, PSME2, PSMB9, JUNB,* and *CEBPD*. As these genes were significantly downregulated in sepsis nonsurvivors, we hypothesize that changes in their expression may signify immune exhaustion in late sepsis and may be good candidates for the sepsis prognosis. However, further

analyses are needed to validate valuable genes whose interplay manifests in sepsis survival.

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Statement of Authorship

All authors contributed to the conceptualization of work, acquisition and analysis of data, drafting and revising, and approved the final version submitted.

Author Disclosure

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