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Postseptic Cognitive Impairment and Expression of APOE in Peripheral Blood: The Cognition After SepsiS (CASS) Observational Pilot Study

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Abstract

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SMB, SJB, ELW, APP, ROH, and MTR designed the study. SMB, ELW, APP, CS, and ROH analyzed and interpreted the data. MTR, SMB, and CM oversaw RNA processing and sample preparation. SMB drafted the report and all other authors revised it for important intellectual content. All authors gave final approval for the manuscript to be published. In order to protect patient privacy and comply with relevant regulations, identified data are unavailable. Requests for deidentified data from qualified researchers with appropriate ethics board approvals and relevant data use agreements will be processed by the Intermountain Office of Research (officeofresearch@imail.org).

Collaborators

Mardee Merrill, Valerie Aston, Katie Brown, Naresh Kumar, Quinn Montgomery. Ethical approval was received and written, informed consent was obtained from participants.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Supplemental Material

Supplemental material for this article is available online.

Background: Cognitive impairment after sepsis is an important clinical problem. Determinants of postseptic cognitive impairment are not well understood. We thus undertook a systems biology approach to exploring a possible role for apolipoprotein E (APOE) in postseptic cognitive impairment.

Design: Prospective, observational cohort.

Setting: Intermountain Medical Center, a tertiary referral center in Utah.

Patients/Participants: Patients with sepsis admitted to study intensive care units.

Interventions: None.

Methods: We obtained peripheral blood for deep sequencing of RNA and followed up survivors at 6 months with a battery of cognitive instruments. We defined cognitive impairment based on the 6-month Hayling test of executive function. In our primary analysis, we employed weighted network analysis. Secondarily, we compared variation in gene expression between patients with normal versus impaired cognition.

Measurements and Main Results: We enrolled 40 patients, of whom 34 were follow-up eligible and 31 (91%) completed follow-up; 1 patient's RNA sample was degraded—the final analytic cohort was 30 patients. Mean Hayling test score was 5.8 (standard deviation 1.1), which represented 20% with impaired executive function. The network module containing APOE was dominated by low-expression genes, with no association on primary analysis ($P = .8$). Secondary analyses suggested several potential lines of future investigation, including oxidative stress.

Conclusions: In this prospective pilot cohort, executive dysfunction affected 1 in 5 survivors of sepsis. The APOE gene was sparsely transcribed in peripheral leukocytes and not associated with cognitive impairment. Future lines of research are suggested.

Keywords

sepsis; cognitive impairment; peripheral blood transcriptome

Introduction

Sepsis, a life-threatening infection associated with organ dysfunction, is a significant public health problem, with an annual incidence of 750 000 in the United States and an associated mortality of 20% to 50%.[1] Clinical advances and research successes over the last 20 years have significantly reduced sepsis mortality.[2] The increasing number of sepsis survivors has allowed researchers to identify substantial, persistent health impairments among survivors of critical illness, including sepsis, now commonly termed postintensive care syndrome.[3]

Sepsis is associated with an acute syndrome of delirium often termed sepsis-associated encephalopathy among the majority of patients.[4,5] Sepsis-associated encephalopathy is an important predictor of later cognitive impairment.[6,7] Once thought to be a temporary problem, there is now mounting evidence that cognitive impairment is a serious and common problem after septic shock. Between 25% and 75% of survivors will be affected by cognitive impairment after hospital discharge.[8,9] Cognitive impairment regardless of etiology is associated with substantial functional, medical, and financial impairments.[10–12]

The causes of postseptic cognitive impairment are not well understood,[13] although some markers of illness severity at hospital discharge do predict cognitive impairment,[14] and a small cohort within a randomized controlled trial suggested that aggressive diuresis ("conservative fluids") may be associated with worse cognitive impairment.[15] Cellular hypoxia, which can occur as a result of hypoxemia, inadequate cardiac output, or microvascular dysfunction, may all contribute.[16] Cognitive impairment may also be related to sedative administration, although data have been equivocal to date.[17,18] An important candidate pathway with promising early results in cognitive impairment caused by other insults is the apolipoprotein pathway,[19,20] although other pathways may also be important. Evidence suggests that while most relevant lipid peroxidation occurs in neuronal tissue, leukocytes (especially monocytes/macrophages[21,22]) express apolipoprotein E (APOE) in multiple disease states.[23–26] We therefore undertook a prospective exploratory study of the peripheral blood transcriptome in patients with sepsis. Some aspects of the study design have been reported elsewhere.[27]

Materials and Methods

Study Design

This prospective, observational study enrolled patients with sepsis admitted to the intensive care units (ICUs) of Intermountain Medical Center, a 450-bed academic referral hospital in Murray, Utah. The study was retrospectively registered with clinicaltrials.gov (NCT03015584), with the primary analysis plan prespecified before any data were reviewed.

Patient Population

We studied adult (≥18 years) patients with sepsis according to SEPSIS-3 definitions (suspected infection and organ dysfunction as manifested by an increase in SOFA score ≥2 over baseline)[1] admitted to a study ICU. Patients were enrolled within 48 hours of ICU admission. We excluded patients with preexisting do not resuscitate/do not intubate orders, primary diagnosis of stroke or intracranial hemorrhage, known preexisting dementia or substantial cognitive impairment of any cause (established from both chart review and/or a score >3 on Informant Questionnaire on Cognitive Decline in the Elderly[28] screening if concern raised from chart review), prior cardiac surgery, known schizophrenia or other psychotic thought disorder, known pregnancy, primary diagnosis of drug overdose, attending physician deemed aggressive care unsuitable, or residence >250 miles from Intermountain Medical Center. Participants who died before 6 months were excluded from analysis.

Patient Data

We collected blood for whole blood RNA deep sequencing at study enrollment and ICU discharge. We also collected demographic, comorbidity, and acute severity of illness data. Outcomes were assessed at hospital discharge, 3, and 6 months, with the primary outcome assessed at 6 months. Outcome measures were administered in person by a trained research coordinator, using standardized instruments. The cognitive battery included verbal fluency (letters F, A, S), digit span (forward and backward) and similarities subtests of the Wechsler Adult Intelligence Scale-IV, Logical Memory I and II from the Wechsler Memory Scale-IV, and the Hayling Sentence Completion Test from the Hayling and Brixton tests.

The Hayling Sentence Completion test (hereafter “Hayling test”) was our primary outcome. The Hayling test is a well-validated measure of executive function[29] and has been used as a key outcome measure in some studies of critical illness survivors.[15,30] Executive function is a central cognitive domain, crucial to overall human function and quality of life,[31,32] and is one of the most commonly impaired cognitive domains in survivors of critical illness and sepsis.[33]

Primary Analysis

For this study, the prespecified primary analysis of the association between the eigengene of the APOE network module from weighted network analysis (WNA; see below) and the 6-month Hayling test was performed. Where a patient had a 3-month follow-up but missed 6-month follow-up, we performed single imputation based on regression of 3-month on 6-month outcomes among other patients. In a sensitivity analysis, we used a linear regression of module eigengene versus Hayling test, controlling for age and sex.

Secondary Analyses

In a post hoc exploratory analysis, we used standard bioinformatics approaches and a false discovery rate limited to 5% to determine which genes were differently expressed between patients with abnormal versus normal Hayling test at 6 months. We used the established threshold of 5.5 to define abnormality in the Hayling test. This analysis was performed to identify potential candidate pathways for future study. In another post hoc exploratory analysis (see Supplementary Material for details), we performed a new WNA after removal of low-expression genes. In another post hoc secondary analysis, we used traditional bioinformatics methods to evaluate differentially expressed genes between patients who were ever delirious (defined as any Confusion Assessment Method [CAM]-ICU score positive during the ICU stay) or never delirious (no positive CAM-ICU score during the ICU stay).

Laboratory Methods

Blood was drawn in PAXgene tubes and immediately frozen at −20°C. We extracted RNA from peripheral whole blood using PAXgene Blood RNA Kit IVD (Qiagen Cat #762164, Germantown, MD) with approximately 1 μg of RNA isolated from whole blood; 100 ng of this RNA was used for Next Generation RNA sequencing (using the Illumina kits with Ribo-Zero Globin processing, 50 cycle single read), aligned to H_sapiens_Feb_2009_B37. Total RNA samples (100-500 ng) were hybridized with Ribo-Zero Globin to substantially deplete both globin RNA and ribosomal RNA species from the samples. Stranded RNA sequencing libraries were prepared as described using the Illumina (San Diego, CA) TruSeq Stranded Total RNA Kit with Ribo-Zero Globin (RS-122-2501 and RS-122-2502). Purified libraries were qualified on an Agilent Technologies (Santa Clara, CA) 2200 TapeStation using a D1000 ScreenTape assay (cat# 5067-5582 and 5067-5583). The molarity of adapter-modified molecules was defined by quantitative polymerase chain reaction using the Kapa Biosystems (Wilmington, MA) Kapa Library Quant Kit (cat#KK4824). Individual libraries were normalized to 10 nmol/L and equal volumes were pooled in preparation for Illumina sequence analysis. Sequencing libraries (25 pmol/L) were chemically denatured and applied to an Illumina HiSeq v4 single read flow cell using an Illumina cBot. Hybridized molecules

were clonally amplified and annealed to sequencing primers with reagents from an Illumina HiSeq SR Cluster Kit v4-cBot (GD-401-4001). Following transfer of the flowcell to an Illumina HiSeq 2500 instrument (HCSv2.2.38 and RTA v1.18.61), a 50-cycle single-read sequence run was performed using HiSeq SBS Kit v4 sequencing reagents (FC-401-4002).

Bioinformatics Analysis

Reads were aligned to the human GRCh38 genome from Ensembl release 87 using STAR version 2.5.2b[34] with splice junctions optimized for 50 base pair reads. Reads were trimmed of adapters and aligned to the reference database using STAR in 2 pass mode to output a BAM file sorted by coordinates. Mapped reads were assigned to annotated genes using feature-Counts version 1.5.1.[35] Normalized counts and differentially expressed genes were identified using DESeq2.[36]

We used DAVID for the pathway analysis and defined significant pathways on the basis of an adjusted *P* value < .05 and a *z*-score whose absolute value was greater than 2.

For the primary prespecified analysis, we used WNA[37] to analyze the transcriptome (details in Supplementary Material). We compared module eigengenes using the Wilcoxon rank-sum statistic. When WNA was uninformative (due to large numbers of low-level transcripts, including APOE) for the primary analysis, we compared expression within transcriptomes between patients with and without cognitive impairment as assessed by the 6-month Hayling test. We used DAVID and R Bioconductor and defined significant pathways on the basis of an adjusted *P* value < .05 and a *z*-score whose absolute value was greater than 2.

To address the risk of type 1 error arising from multiple comparisons, where applicable, we limited the false discovery rate to <5% using the technique of Benjamini and Hochberg.[38] We performed analyses in R version 3.2.3 (www.r-project.org).[39]

Sample Size

Although this study was primarily intended to generate hypotheses and provide relevant effect estimates for subsequent studies, we estimated that 40 enrolled patients with a 12% mortality (based on local experience[40]) and a 6% loss to follow-up (based on prior experience[41]) would provide 33 patients for follow-up. Assuming a 40% incidence of cognitive impairment (ie, 13 patients with impairment, 20 without impairment), for RNA-seq, we had 80% power to detect a 1.5-fold change in gene expression with a 0.05 proportion of differentially expressed genes at a 0.05 false discovery level with average standard deviations of ≤0.26 on the natural log scale. For binary predictors, we had 80% power ($\alpha = 0.05$) to detect an approximately 60% absolute difference in proportions (ie, 5% vs 65%). Power calculations were performed in PASS v. 12[42] and R (ssize.fdr).[39]

Ethical Considerations

The Intermountain Medical Center institutional review board approved this study. All patients or their surrogates provided written informed consent. Where possible, patient reconsent was obtained after initial surrogate consent. A deidentified file of gene count

(log2-normalized) data and relevant covariates is provided as a Supplementary Material to this manuscript.

Results

As outlined in Figure 1, we screened 631 patients, of whom 105 met eligibility criteria. Among those 105 patients, we enrolled 40 patients. One withdrew from the study before any study procedures and requested that data not be collected; 1 withdrew from the study after baseline data collection, and 4 (11%) died before follow-up. Among 34 follow-up-eligible patients, 31 (91%) had the primary outcome measured (N = 30) or imputed from a regression on 3-month outcomes (N = 1). One patient was excluded from analysis based on low-quality RNA after extraction. The analytic data sample was thus 30 patients. Table 1 displays the distribution of relevant predictors and hospital outcomes on all patients. Table S1 in the Supplemental Material displays baseline characteristics among enrolled patients and the subset within the analytic cohort.

Among the 30 analyzed patients, the mean Hayling test score was 5.8 (standard deviation 1.1); 6 (20%) had impairment of executive function as measured by an abnormal Hayling result.

Weighted network analysis identified the network module containing APOE, which was the largest module and was dominated by low expression transcripts. The gene ontology terms within relevant WNA modules are displayed in Table S2 of the Supplementary Material. Distribution of module eigengenes by cognitive impairment is displayed in Table S3 of the Supplementary Material. The eigengene of the module containing APOE was not associated with cognitive impairment as measured by an abnormal 6-month Hayling test ($P = .6$). A sensitivity analysis using linear regression of the raw 6-month Hayling test, controlling for age and sex, also did not identify a significant association with the APOE module eigengene ($P = .8$). No other network module showed significant association with the Hayling scores after correction for multiple comparisons. More complete results of WNA, including module membership for differentially expressed genes (Table S4), are reported in the Supplementary Material.

In our post hoc exploratory analysis using traditional bioinformatics methods, we identified 228 significant genes between abnormal versus normal including 54 upregulated and 174 downregulated genes. A heatmap comparing gene expression from the top 20 up- and downregulated genes and a volcano plot are displayed in Figure 2A and B.

Several transcripts clustered into multiple biological pathways that were suggested to be differentially activated or suppressed on the basis of Gene Set Enrichment Analysis (Table S5 in the Supplementary Material).[43] Pathways included those related to general cellular function (eg, ribosome, proteasome, and oxidative phosphorylation) and specific disease states (eg, Parkinson disease, Alzheimer disease, and Huntington disease). Some of the potential genes of interest include the immune molecules CCR1 and TREML4 (lower expression in cognitive impairment) or the lipid-modulating gene ACP6 (higher expression in cognitive impairment). In the secondary analysis removing low-expression genes, module

eigengenes were not significantly associated with outcome (Table S6 in the Supplementary Material) but an association with oxidative stress was observed (Supplemental results and Tables S7 and S8 in the Supplementary Material).

In the secondary analysis using delirium in the ICU as the clinical end point, only 1 gene was statistically significant (adjusted *P* value .04): sidekick cell adhesion molecule 1 (SDK1). The SDK1 gene had higher expression (slightly more than double the expression, 1.2 log2-fold increased expression) in patients with delirium during their ICU stay.

Discussion

In this prospective pilot study of patients having sepsis with 6-month follow-up for cognitive function, we did not identify a relationship between APOE gene transcription in peripheral blood and postseptic cognitive impairment nor did we identify other meaningful signals in canonical gene pathways on our primary analysis.

Our study has several advantages. We used a well-validated method for identification of postseptic cognitive impairment and had excellent cohort retention (>85%). We enrolled patients prospectively, with timely preservation of RNA and unbiased measurement of the total transcriptome. We used standard techniques for transcriptome analysis. We also strictly delineated prespecified primary analysis from our subsequent exploratory analysis and limited our claims about candidate transcripts in the exploratory analysis.

This study was performed as a pilot study to determine whether to undertake further investigations of APOE within the peripheral blood transcriptome. The primary limitation of our study is its small size and the attendant risk of type II statistical error (failing to identify a relationship that truly exists). Although the septic transcriptome has been shown to vary based on age, we did not have a sufficient sample size to robustly control for age in our analyses.[44] Although there was no promising early signal for APOE, the peripheral transcriptome may provide insights into cognitive function in larger samples, perhaps following the pathways, including oxidative stress, suggested in exploratory analyses. The fact that expression was so consistently low across all patients does argue against a relevant role for APOE expression in peripheral leukocytes. We prefer to publish these data in order to mitigate the risk of publication bias leading to overemphasis on positive results.[45,46]

The rationale for exploring APOE has been reasonably strong. Apolipoprotein E is a lipid-trafficking molecule that is closely associated with Alzheimer disease,[47] and other patterns of progressive cognitive decline.[48,49] Polymorphisms in APOE, especially the ∈4 variant, have been associated with cognitive impairment after traumatic brain injury,[50] (albeit with conflicting information[50–53]) as well as carbon monoxide poisoning and general critical illness.[54,55] Lipid peroxidation may play a particularly important role in cognitive impairment after sepsis,[56,57] and APOE appears to be related to the burden of lipid peroxidation in experimental models.[56] Early human data suggest that circulating APOE levels are associated with sepsis per se,[58,59] probably reflecting the importance of APOE to presentation of lipid antigens from invading microorganisms.[60] Our results are limited by our use of RNA transcripts in peripheral blood cells, while APOE expression is likely

more important in other cell lines, especially neurons. However, neurons are unavailable for analysis in individuals in whom cognition can be measured. We analyzed the peripheral transcriptome given the safety and accessibility of those cells and potential relevance in other disease states;[21–26] we cannot comment on the role of APOE expression in postseptic cognitive impairment in other cell types. We also note as a limitation that we did not explore genetic polymorphisms within APOE, but rather messenger RNA expression levels in peripheral leukocytes. It is possible that APOE gene variants are in fact associated with sepsis-associated cognitive impairment; future work should address that question.

Conclusions

In a prospective pilot cohort of patients with sepsis admitted to the ICU, APOE expression levels were low in peripheral leukocytes and appeared unrelated to executive cognitive function at 6 months after sepsis hospitalization. Other avenues than peripheral transcriptomics should likely be undertaken to further interrogate the relationship between APOE (including its polymorphisms) and sepsis-associated cognitive impairment. Other lines of inquiry, including oxidation, are suggested.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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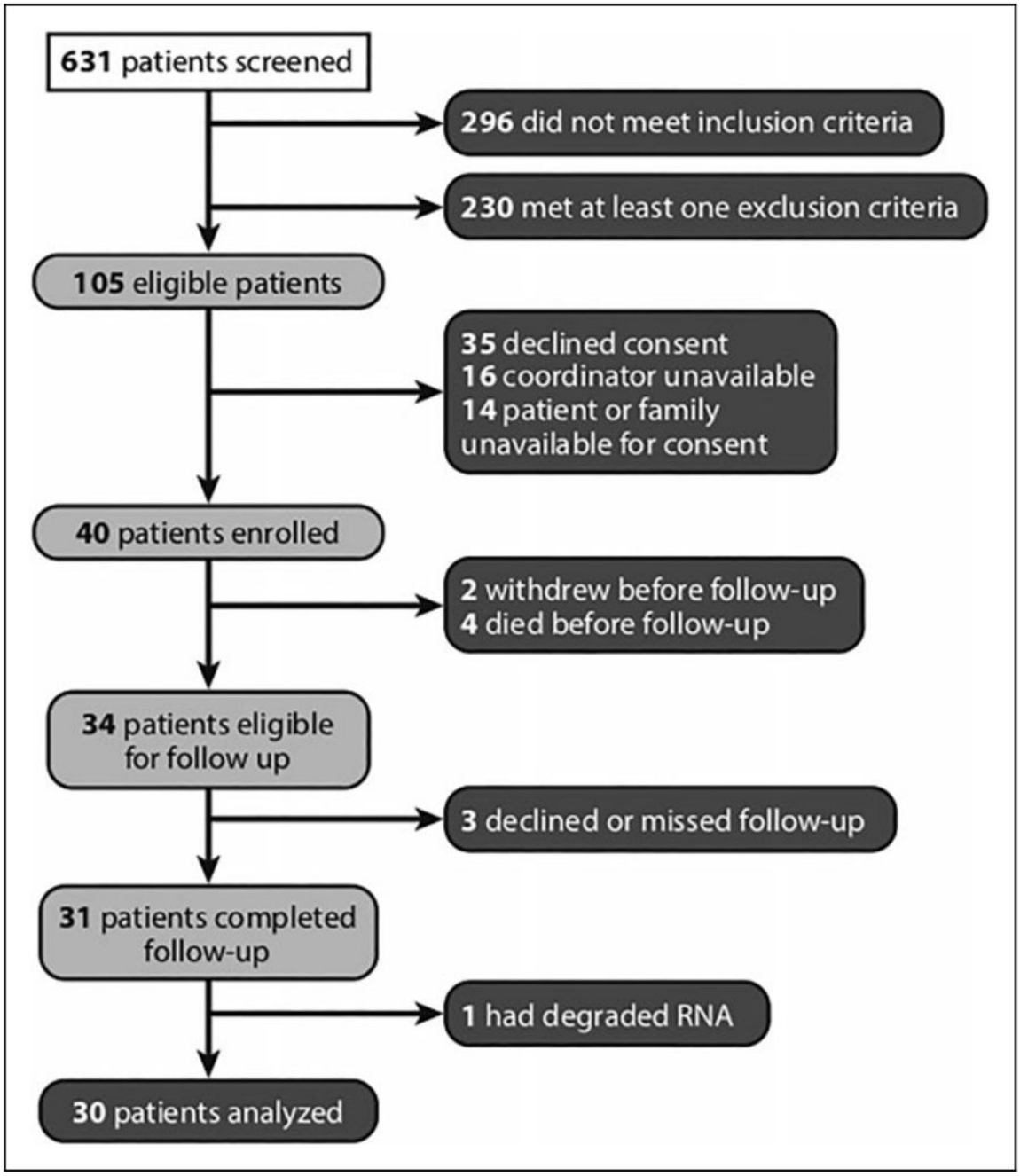

Figure 1.
Patient flow diagram depicting the identification of patients for this study.

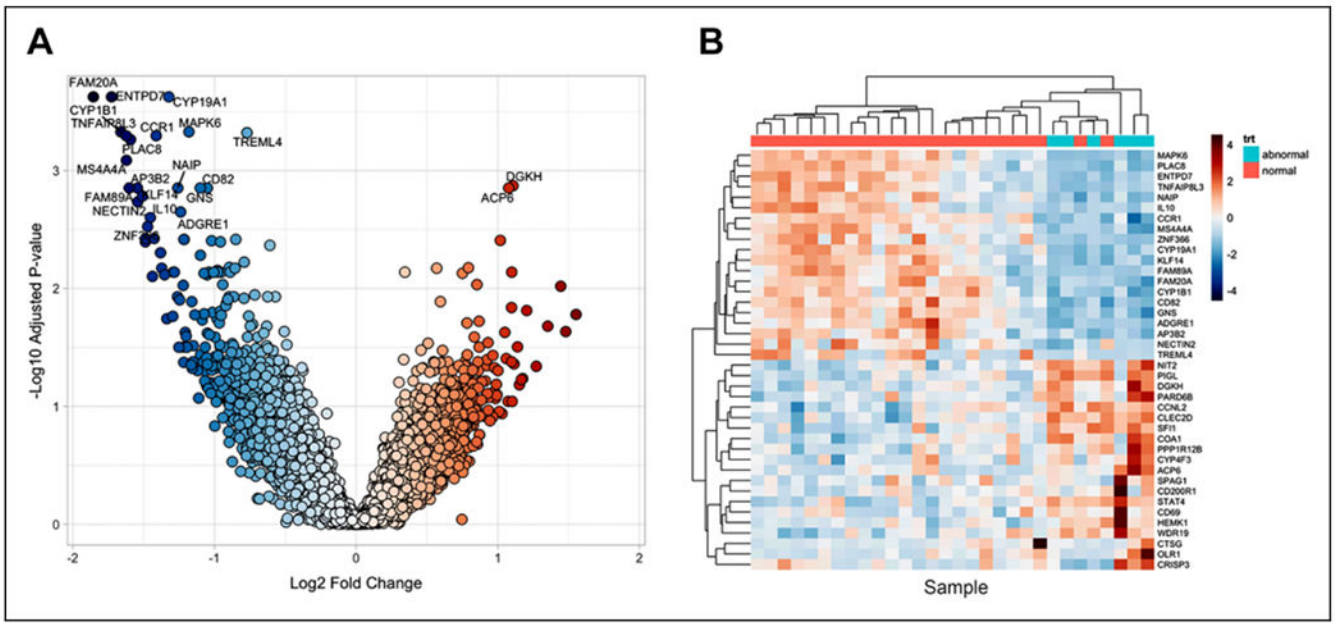

Figure 2.

A, Volcano plot of differentially expressed genes. B, Heatmap of gene expression for top 20 up- and downregulated transcripts, by participants.

Table 1.

Characteristics of the Final Study Population.[a]

Variable	Entire Analytic Cohort (N = 30)	With Cognitive Impairment (N = 6)	Without Cognitive Impairment (N = 24)
Demographics			
Age	56 (47-69)	62 (51-68)	54 (47-68)
Race			
American Indian or Alaskan Native	0 (0%)	0 (0%)	0 (0%)
Asian	1 (3%)	0 (0%)	1 (4%)
Black	0 (0%)	0 (0%)	0 (0%)
Native Hawaiian or other Pacific Islander	1 (3%)	0 (0%)	1 (4%)
White	28 (93%)	6 (100%)	22 (92%)
Female sex	17 (57%)	2 (33%)	15 (62%)
Peak educational attainment			
Less than high school	3 (10%)	2 (33%)	1 (4%)
High school completed	7 (23%)	1 (17%)	6 (25%)
Some college	10 (33%)	2 (33%)	8 (33%)
Associate degree	2 (7%)	1 (17%)	1 (4%)
Bachelor degree	5 (17%)	0 (0%)	5 (21%)
Master, doctorate, or other professional degrees	3 (10%)	0 (0%)	3 (13%)
Median income for census block	71 390 (55 038-90 932)	56 058 (51 221-68 828)	74 209 (60 006-93 961)
Baseline living arrangement			
Home	27 (90%)	5 (83%)	22 (92%)
Hospital ward	1 (3%)	0 (0%)	1 (4%)
Skilled nursing facility	1 (3%)	1 (17%)	0 (0%)
Rehabilitation facility	1 (3%)	0 (0%)	1 (4%)
Assisted living facility	0 (0%)	0 (0%)	0 (0%)
Baseline employment status			
Working full time (at least 32 hours/week)	11 (37%)	2 (33%)	9 (38%)
Working part time	2 (7%)	0 (0%)	2 (8%)
Unemployed and looking for work	2 (7%)	0 (0%)	2 (8%)
Homemaker	3 (10%)	0 (0%)	3 (13%)

Variable	Entire Analytic Cohort (N = 30)	With Cognitive Impairment (N = 6)	Without Cognitive Impairment (N = 24)
Retired	9 (30%)	2 (33%)	7 (29%)
Receiving disability payments	2 (7%)	2 (33%)	0 (0%)
Other	1 (3%)	0 (0%)	1 (4%)
Comorbidities and risk factors			
Comorbidities			
Congestive heart failure	3 (10%)	1 (17%)	2 (8%)
Depression requiring treatment	6 (20%)	0 (0%)	6 (25%)
Anxiety requiring treatment	7 (23%)	0 (0%)	7 (29%)
Chronic kidney failure requiring dialysis	2 (7%)	1 (17%)	1 (4%)
Diabetes mellitus	8 (27%)	3 (50%)	5 (21%)
Ever drink alcohol	13 (43%)	0 (0%)	13 (54%)
Current smoker	6 (20%)	1 (17%)	5 (21%)
Antidepressant medications	7 (23%)	0 (0%)	7 (29%)
Narcotic pain relievers	9 (30%)	3 (50%)	6 (25%)
Mood stabilizers	2 (7%)	0 (0%)	2 (8%)
Acute illness attributes			
Admission APACHE II	18 (13 - 24)	20 (16 - 26)	18 (13 - 24)
Admission SOFA score	8 (5 - 11)	6 (5 - 10)	8 (6 - 11)
Days of delirium	0 (0 – 1)	0 (0 – 1)	0 (0 – 1)
Ever delirious	7 (23%)	2 (33%)	5 (21%)
Ever mechanically ventilated	7 (23%)	1 (17%)	6 (25%)
Ever on vasopressor agents	10 (33%)	1 (17%)	9 (38%)
Sepsis etiology			
Pneumonia	11 (37%)	0 (0%)	11 (46%)
Urosepsis	7 (23%)	1 (17%)	6 (25%)
Central nervous system	0 (0%)	0 (0%)	0 (0%)
Unknown	3 (10%)	1 (17%)	2 (8%)
Catheter related	0 (0%)	0 (0%)	0 (0%)
Abdominal	4 (13%)	1 (17%)	3 (13%)
Skin and soft tissue	5 (17%)	3 (50%)	2 (8%)
Endocarditis/bacteremia	0 (0%)	0 (0%)	0 (0%)

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Variable	Entire Analytic Cohort (N = 30)	With Cognitive Impairment (N = 6)	Without Cognitive Impairment (N = 24)
ICU-free days to day 28	25 (23-26)	25 (24-26)	24 (24-26)
ICU length of stay	3.0 (1.6-4.0)	2.3 (1.3-3.8)	3.3 (1.7-4.0)
Hospital length of stay	5.6 (3.9-8.9)	6.0 (4.3-9.8)	5.3 (3.9-8.4)
Discharge destination			
Home	24 (80%)	4 (67%)	20 (83%)
Nursing home	5 (17%)	2 (33%)	3 (13%)
Rehabilitation facility	1 (3%)	0 (0%)	1 (4%)
Hayling at 6 months	6 (6-6)	4.5 (4-5)	6 (6-6)

Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; ICU, intensive care unit; IQR, interquartile range; SOFA, Sequential Organ Failure Assessment.

[a]Median (IQR), N (%).