HHS Public Access

Author manuscript

Shock. Author manuscript; available in PMC 2024 September 01.

Published in final edited form as:

Shock. 2023 September 01; 60(3): 362–372. doi:10.1097/SHK.0000000000002176.

Pathways Associated with Positive Sepsis Survival Outcomes in African American/Black and Non-Hispanic White UTI Patients

Kathryn L. Kapp[a,b], **Min Ji Choi**[a], **Kun Bai**[c], **Liping Du**[c,d], **Sachin Yende**[e,f,g], **John A. Kellum**[f], **Derek C. Angus**[e,f,g], **Octavia M. Peck-Palmer**[e,f,g,h], **Renã A. S. Robinson**[a,b,*]

[a]Department of Chemistry, Vanderbilt University, Nashville, TN, 37235, USA

[b]The Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, TN, 32732, USA

[c]Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN, 37203, USA

[d]Vanderbilt Center for Quantitative Sciences, Vanderbilt University Medical Center, Nashville, TN, 37232, USA

[e]The Clinical Research, Investigation, and Systems Modeling of Acute Illnesses (CRISMA) Center, University of Pittsburgh, Pittsburgh, PA, 15213, USA

[f]Department of Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15213, USA

[g]Department of Clinical and Translational Science, University of Pittsburgh, PA, 15261, USA

[h]Department of Pathology, University of Pittsburgh, Pittsburgh, PA, 15213, USA

Abstract

Urinary tract infections (UTIs) are a common cause of sepsis worldwide. Annually, over 60,000 US deaths can be attributed to sepsis secondary to UTIs, and African American/Black adults have higher incidence and case-fatality rates more so than Non-Hispanic White adults. Molecular-level factors that may help partially explain differences in sepsis survival outcomes between African American/Black and Non-Hispanic White adults are not clear. In this study, patient samples (N = 166) from the Protocolized Care for Early Septic Shock (ProCESS) cohort were analyzed using discovery-based plasma proteomics. Patients had sepsis secondary to UTIs and were stratified according to self-identified racial background and sepsis survival outcomes. Proteomics results suggest patient heterogeneity across mechanisms driving survival from sepsis secondary to UTIs. Differentially-expressed proteins (N = 122, FDR-adjusted $p < 0.05$) in Non-Hispanic White sepsis

[*]Author to whom correspondence should be addressed: Prof. Renã A. S. Robinson, Ph.D., Dorothy J. Wingfield Phillips Chancellor's Faculty Fellow, Department of Chemistry, Vanderbilt University, 5423 Stevenson Center, Nashville, TN 37235, Fax: 615-343-1234, Tel: 1-615-343-0129, rena.as.robinson@vanderbilt.edu.

AUTHOR CONTRIBUTIONS

RASR conceived the project idea and design and provided study oversight. KLK and RASR designed the proteomics experiments. KLK prepared the samples and performed the data acquisition, processing, and quality control monitoring. RASR and KLK both contributed to data interpretation and manuscript drafts. MJC assisted with sample preparation. KB and LD contributed to statistical analysis. JAK, SY, OMPP, and DCA shared data from ProCESS. All authors reviewed the manuscript.

CONFLICTS OF INTEREST

Vanderbilt University and the University of Pittsburgh have jointly filed for patent protection for a panel of biomarkers arising from related work.

JAK holds stock in and is currently a full-time employee of Spectral Medical, Toronto, ON, and discloses consulting fees paid by Astute Medical, a BioMerieux company. Other authors declare no competing interests.

survivors were primarily in immune system pathways, while differentially-expressed proteins (N = 47, FDR-adjusted $p < 0.05$) in African American/Black patients were mostly in metabolic pathways. However, in all patients, regardless of racial background, there were 16 differentially-expressed proteins in sepsis survivors involved in translation initiation and shutdown pathways. These pathways are potential targets for prognostic intervention. Overall, this study provides information about molecular factors that may help explain disparities in sepsis survival outcomes among African American/Black and Non-Hispanic White patients with primary UTIs.

Keywords

Sepsis; survivor; race; ethnicity; plasma; proteomics; urinary tract infection; African American/Black

INTRODUCTION

Urinary tract infections (UTIs) are common infections worldwide and in the United States, with an estimated 150 million cases worldwide each year.[1,2] Higher prevalence of UTIs is associated with increased age and female sex, with ~50% of adult women experiencing at least one UTI in their lifetime.[1,3] Recurring infections are also common in women.[1] UTIs, usually caused by uropathogenic *Escherichia coli* (UPEC),[2] can affect healthy individuals or patients that require catheter usage.[2,4,5] UTIs can progress to sepsis and septic shock when not properly treated, greatly increasing patient mortality rates.[6–8]

Racial and ethnic disparities exist in UTIs[9,10] and sepsis.[11–15] Non-White patients have higher odds of developing catheter-associated UTIs than White patients.[9] Black women have reported lower odds of recurrent UTIs compared to White women, but this may be attributed to underestimates of diagnoses in Black women.[10] African American/Black UTI patients have higher incidence and case-fatality rates from genitourinary sepsis than Non-Hispanic White UTI patients.[16] Disparities in sepsis mortality rates have been demonstrated in cohorts ranging from ~200,000 to ~2.5 million patients, with patient data as recent as 2018.[11,13,15,17] Interestingly, a recent study by Kokoefer *et al.* did not find differences in hospital or intensive care unit mortality across racial/ethnic backgrounds but did find that African American sepsis patients were younger than and had higher creatinine and lower hemoglobin levels than Non-Hispanic White sepsis patients.[18] Socioeconomic factors, access to and quality of healthcare, systemic racism, and chronic disease burden are partial contributors to disparities in sepsis, but notably, differences in patient mortality rates still exist after accounting for these factors.[12,15,17,19–22] Therefore, disparities in sepsis survival outcomes are likely related to socioeconomic and biological factors.

Various studies have suggested molecular-level responses that collineate with racial/ethnic backgrounds and the host immune response, particularly in zinc finger, metal binding, and iron homeostasis genes;[23] inflammatory and coagulation factors;[24–26] and apolipoprotein L1 alleles.[27,28] More generally, proteins and metabolites in immune response, coagulation, metabolism, and cellular assembly and movement pathways differ between survivors and non-survivors in primarily Non-Hispanic White patients with community-acquired pneumonia (CAP).[29–38]

Our laboratory previously demonstrated similarities and differences in the plasma proteome of African American/Black and Non-Hispanic White survivors of sepsis secondary to intra-abdominal infections.[39] Proteins related to hepatic fibrosis/hepatic stellate cell activation, such as vascular cell adhesion molecule 1 (VCAM1), were decreased in survivors of both racial groups and suggested universal changes in sepsis patients.[39] However, proteins related to the complement system and T-cell activation and proliferation were only significantly represented in African American/Black survivors.[39] The expression levels of several acute phase and complement proteins, as well as proteins associated with type I interferon binding and receptor activity and natural killer-cell mediated cytotoxicity, were associated with both the racial background of the patient and sepsis survival outcome.[39] However, sepsis mortality rates differ based on primary infection source, especially between intra-abdominal infections and UTIs.[40] Transcriptomics and metabolomics analyses have suggested a source-specific host response to sepsis.[41,42]

Here, we are interested in better understanding the molecular response to UTIs and how this response is related to sepsis survival outcomes in patients with different racial backgrounds. The initial insight from this study may partially explain disparities in sepsis survival outcomes. Quantitative proteomics was applied to plasma samples from African American/Black and Non-Hispanic White patients (N = 166) enrolled in the Protocolized Care for Early Septic Shock (ProCESS) cohort, a randomized trial comparing fluid resuscitation strategies in septic shock patients across 31 academic US hospitals.[43] Various treatment strategies showed equal effectiveness on patient mortality.[43] The ProCESS cohort included diversity with regards to geographical location, patients' racial and ethnic background, and primary cause of sepsis.

MATERIALS AND METHODS

Ethics Statement, Study Design, Patients, & Plasma Collection

ProCESS was a multicenter trial comparing fluid resuscitation strategies for septic shock from March 2008 to May 2013.[43] Across 31 US academic emergency departments (EDs), 1,341 patients were enrolled in the trial.[43] Details and eligibility criteria have previously been described,[43,44] and the Institutional Review Boards (IRBs) at the University of Pittsburgh and all 31 hospitals approved.

For this sub-analysis, which was approved by the Vanderbilt University IRB, ProCESS patients were selected based on self-identified race (African American/Black or Non-Hispanic White), primary infection source (UTI),[44] and availability of plasma. A total of N = 166 patients were selected for this study. Plasma samples were collected at "time zero," which referred to the point of ED admission and enrollment in the ProCESS cohort prior to treatment, and survival outcomes refer to a patient's status 90 days post-ED admission.[39] Treatment group was not included as a covariate in this study.[43]

Plasma samples were analyzed using a bottom-up, quantitative proteomics workflow in a randomized and blinded fashion. The workflow featured immunodepletion, automation of several sample preparation steps, and tandem mass tag (TMTpro) labeling. Following TMTpro labeling, peptides were fractionated with high pH reverse-phase (RP) fractionation

and further separated and detected with liquid chromatography-tandem mass spectrometry (LC-MS/MS; Figure 1A). An overview is described below and details were described previously.[39] Selected proteins from our workflow have previously been validated via Western blotting.[39]

Sample Depletion & Digestion

Two 40-µL aliquots of crude plasma were injected onto a multiple affinity removal system Hu-14 (MARS-14) column (Agilent; Santa Clara, CA, USA) to deplete the top 14 most abundant plasma proteins. Immunodepletion was performed following the manufacturer's protocol with quality control (QC) samples run daily (*unpublished results*). The two unbound fractions were concentrated, combined, and frozen at −80 °C until further analysis. A bicinchoninic acid (BCA) protein assay (Thermo Fisher Scientific; Waltham, MA, USA) was used to determine protein concentration. A QC sample was made by pooling equimolar amounts of each sample (N = 166) at the protein level.

Using a Biomek i7 liquid handling workstation (Beckman Coulter; Atlanta, GA, USA), in-solution digestion of protein (60 µg) was performed in 100 mM ammonium bicarbonate buffer for each sample. Protein was reduced with 200 mM dithiothreitol, alkylated with 200 mM iodoacetamide, and digested with trypsin/Lys-C at a 50:1 sample:enzyme ratio. Digested samples were desalted with a C18 filter plate and a positive pressure apparatus, dried using centrifugal evaporation, and stored at −80 °C until further analysis.

Sample Tagging

Samples were randomly assigned to 12 TMTpro batches, with each batch containing one QC sample and at least one sample from each study group for a total of 15 patient samples (see Supplemental Table S1 for batch assignments). Each batch was balanced for survival outcome, racial/ethnic background, and age. Peptide samples (25 µg) were reconstituted in 100 mM triethyl ammonium bicarbonate and labeled with a TMTpro reagent following the manufacturer's protocol in a 1:8 sample:tag ratio (Thermo Fisher Scientific; Waltham, MA, USA). Samples in each batch were pooled, acetonitrile (ACN) solvent was removed with centrifugal evaporation, and peptides were desalted as previously described.[39] Pooled TMTpro batches (295 µg) were fractionated using basic RPLC offline fractionation to a final set of 24 concatenated fractions.[39,45] Each fraction (1 µg) was reconstituted in LC-MS grade water with 0.1% formic acid (FA) and stored at −80 °C.

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Acquisition

LC-MS/MS analysis was performed as previously described,[39] but with a shorter LC gradient. Each fraction (1 µg) was injected in duplicate onto a Dionex UltiMate 3000 LC system coupled to a Q-Exactive HF mass spectrometer (both Thermo Fisher Scientific; Waltham, MA, USA) operated in positive mode. Peptides were loaded onto a commercial C18 trap column (75 µm i.d. x 2 cm, 100 Å, 3 µm; Thermo Fisher Scientific, Waltham, MA, USA) prior to separation on an in-house C18 packed column (100 µm i.d. x 30 cm, 100 Å, 2.5 µm) with a pulled tip over the following 160 min gradient: 0–2 min, 2% B; 2–5 min, 2–7% B; 5–88 min, 7–16% B; 88–133 min, 16–25% B; 133–140 min, 25–85% B; 140–146

min, 85% B; 146–148 min, 85–4% B; 148–160 min, 4% B. Mobile phase A was 0.1% FA and mobile phase B was 0.1% FA in ACN.

Data Analysis

RAW files were analyzed using Proteome Discoverer v2.5 software (Thermo Fisher Scientific; Waltham, MA, USA). All technical replicates and fractions from each batch were combined into one file and searched against the UniProt human reviewed protein database (02/04/22, 42,264 sequences) using SEQUEST-HT. Selected protein groups will be referred to as proteins in the rest of this analysis. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org/) via the PRIDE[46] partner repository with the dataset identifier PXD039509. Peptides were filtered to only include those identified with high confidence and their corresponding proteins, which was further limited to those with at least two peptide spectral matches. TMTpro reporter ion intensities of quantified proteins were normalized both within and across batches,[47,48] and normalized data were used for further statistical analysis.

Linear regression methods were used to identify protein factors associated with survival and race. For each protein factor, intensity was the dependent variable, and two regression models were fitted: one with 90-day survival (yes or no) as the independent variable, and one with survival outcome, race, and their interaction as independent variables. Robust standard errors were used to account for batch clusters, and all models were adjusted for age and sex.

We excluded proteins (N = 809) with reporter ion values missing in QC channels and/or proteins that were detected in ≤20 samples (<12% of all 166 samples) due to small sample size. P-values were adjusted using the Benjamini-Hochberg (B-H) false discovery rate (FDR) procedure to account for multiple comparisons, and adjusted p-values < 0.05 were considered statistically significant. Analyses were performed using R version 4.2.1 and the rms package.[49]

Fold change (FC) values were calculated for each protein by dividing the mean of sepsis survivors' TMTpro levels by the mean of sepsis non-survivors' TMTpro levels. FC cutoffs were established using a power analysis strategy previously described:[50] ≥1.62 or ≤0.62 for comparisons of survivors vs. non-survivors; ≥1.76 or ≤0.57 for comparisons of Non-Hispanic White survivors vs. non-survivors; and ≥2.51 or ≤0.40 for comparisons of African American/Black survivors vs. non-survivors. These cutoffs correspond to the protein for which the smallest number of patients in that subgroup had reporter ion values.

Ingenuity Pathway Analysis (IPA) and Search Tool for Retrieval of Interacting Genes/Proteins (STRING)[51] were used to identify significant biological pathways and functions (B-H corrected $p < 0.05$ and minimum interaction score = 0.400, respectively).

RESULTS

Patient Characteristics

Demographic characteristics of the subset of ProCESS patients (N = 166) selected for this study are shown in Table 1. Most patients (74%) survived from sepsis secondary to UTIs 90 days post-ED admission, and ~27% of the patients in this study self-identified as African American/Black. Notably, African American/Black sepsis survivors had a significantly lower percentage of females (34%) than the other three study groups (64–80%; $\chi^2 = 15.271$). Survivors were 6–11 years younger than non-survivors, and notably, African American/Black non-survivors were five years younger than Non-Hispanic White non-survivors (68 years vs. 73 years, respectively). Differences in patient age were driven by survival outcome ($p = 0.012$) and not racial background ($p = 0.470$). There was no difference in the frequency of comorbidities such as diabetes and renal disease across patients.

Differentially-Expressed Proteins & Pathways Associated with Survival

The proteomics workflow subjected ProCESS patient plasma samples to a bottom-up approach relying on LC-MS/MS (Figure 1A). An initial 3,219 proteins were identified (183,725 peptides), and of those, 2,410 proteins (175,511 peptides) were analyzed with a linear regression model (survival only model; Figure 1B). After FDR adjustment (adjusted $p < 0.05$), 248 proteins had significant differences in relative protein levels between sepsis survivors and non-survivors across all patients (Figure 1B, Supplemental Figure S1A, and Supplemental Table S2).

Synaptogenesis signaling was the most significantly altered pathway between survivors and non-survivors (B-H $p = 0.006$), and germ cell-Sertoli cell junction signaling, eukaryotic initiation factor 2 (EIF2) signaling, and tight junction signaling (all B-H $p = 0.025$) were also altered (Supplemental Figure S1B). Other significant pathways associated with survival outcome broadly relate to inflammation, the endothelial system, and metabolism (Supplemental Figure S1B). Similarly, in STRING analyses, cell adhesion and immune processes (such as complement and T-cell regulation) were highly enriched, as well as metabolic (e.g., fatty acid) and calcium ion binding functions (*data not shown*). The most enriched cellular components included proteins localized to immune cells, actin filament bundles, and neuromuscular junctions. Cadherin-17 (CDH17), myristoylated alanine-rich C-kinase substrate (MARCKS), VCAM1, and adapter molecule crk (CRK) are examples of proteins decreased in sepsis survivors (Supplemental Figure S1A). Acute phase protein histidine-rich glycoprotein (HRG) was the only differentially-expressed protein increased in sepsis survivors.

Differentially-Expressed Proteins & Pathways Associated with Survival within Racial Groups

We assessed survival outcome from within African American/Black and Non-Hispanic White patient groups using a linear regression model with survival outcome and race as independent variables for 2,410 proteins. After FDR adjustment (adjusted $p < 0.05$), 122 (5.1% of the proteins in regression) and 47 (2.0%) proteins were differentially-expressed between sepsis survivors and non-survivors among Non-Hispanic White or

African American/Black patients, respectively (Supplemental Table S3; Figures 2A and 2B). Protein ELYS and interleukin (IL)-5 receptor subunit α (both adjusted $p < 0.001$; Figure 2A) were higher in Non-Hispanic White survivors compared to non-survivors. HRG and immortalization up-regulated protein (adjusted $p = 0.003$ and 0.007, respectively; Figure 2B) were higher in African American/Black survivors compared to non-survivors.

Differentially-expressed proteins associated with survival outcome across African American/Black and Non-Hispanic White patients include examples such as: 60S ribosomal protein L12 (FC = 0.05 and 0.30, respectively); lysine-tRNA ligase (FC = 0.05 and 0.33, respectively); 40S ribosomal proteins S3 (FC = 0.04 and 0.41, respectively) and S21 (FC = 0.09 and 0.51, respectively); inositol polyphosphate 1-phosphatase (FC = 0.06 and 0.41, respectively); heterogeneous nuclear ribonucleoprotein L (FC = 0.04 and 0.44, respectively); and X-ray repair cross-complementing protein 6 (FC = 0.10 and 0.40, respectively). For these example proteins, p-values were < 0.001 in both groups, with the exception of 40S ribosomal protein S3 in Non-Hispanic White patients ($p = 0.048$).

Differentially-expressed proteins (N = 17) whose abundances were lower in survivors compared to non-survivors across Non-Hispanic White and African American/Black patients (Figure 2C) were primarily ribosomal proteins or ribonucleoproteins involved in translation and metabolic pathways. These proteins are localized to the urogenital system and female reproductive organs. Overall, most differentially-expressed proteins that were associated with positive survival outcomes were identified within a single group (Figure 2C), and the abundances of these proteins were primarily lower in sepsis survivors compared to non-survivors.

Differentially-expressed proteins (N = 122) in Non-Hispanic White patients were associated with survival outcome and mapped to several enriched biological processes and molecular functions in STRING (Supplemental Table S4), including cell adhesion, calcium binding, and the cytokine response. Enriched STRING clusters included IL signaling; Janus kinase-signal transducer and activator of transcription (JAK-STAT) gene and protein expression following IL-12 stimulation; selenoamino acid metabolism; and extracellular matrix (ECM) organization. These pathways have been reported as functional in immune cells (i.e., macrophages and neutrophils), actin bundles of the cytoskeleton, the ECM, and polyribosomes. However, no canonical pathways were significantly altered between Non-Hispanic White sepsis survivors and non-survivors in IPA (Figure 2D).

Differentially-expressed proteins in African American/Black patients (N = 47) associated with survival outcome were significantly involved in EIF2 signaling, telomere extension, and stearate biosynthesis I via IPA (Figure 2D). These findings suggest DNA replication, protein translation, and metabolism may influence positive patient survival outcomes from sepsis in this group. Correspondingly, many metabolic and catabolic processes were enriched in STRING (Supplemental Table S4), as well as the following Reactome pathways: eukaryotic translation termination, peptide chain elongation, selenoamino acid metabolism, and selenocysteine synthesis.

Proteins with Significant Race-Survival Outcome Interaction

To further assess protein changes across sepsis patients, we included a race-survival outcome interaction term in the linear regression model with race as an independent variable. Seventy-one (2.9% of the proteins in regression) proteins had a significant race-survival outcome interaction (adjusted $p < 0.05$; Table 2). EIF2 signaling was a significant canonical pathway, and STRING analysis revealed involvement in tumor necrosis factor (TNF) response, translation, and metabolism pathways (Figure 3; Supplemental Table S5). Across our within-group analyses (Figure 2) and this race-survival outcome interaction term (Table 2), 16 proteins overlapped (Figure 4). Similar trends in expression levels were observed across these 16 proteins in sepsis survivors and non-survivors from both racial groups. Notably, the abundance levels for all these proteins were highest in African American/Black sepsis non-survivors. These proteins were primarily involved in EIF2 signaling, but other translation and metabolic pathways were also implicated (Figure 5).

DISCUSSION

Molecular changes associated with sepsis survival from patients with primary UTIs are not known. A prior proteomic study in UTI models and patients who developed sepsis proposed lipopolysaccharide-binding protein, clusterin, and VCAM1 as diagnostic markers of sepsis.[52] Others have identified differentially-expressed proteins involved in coagulation, the acute phase response, lipid homeostasis, and iron ion transport as associated with sepsis severity.[53] Our laboratory previously used plasma proteomics to study sepsis survival in a diverse cohort of patients with primary intra-abdominal infection, identifying proteins related to hepatic fibrosis/hepatic stellate cell activation (e.g., VCAM1) that were decreased in sepsis survivors.[39] However, proteins related to the complement system and T-cell activation and proliferation were significantly represented in African American/Black survivors, and the expression levels of several acute phase and complement proteins were dependent on survival outcome and self-identification within African American/Black or Non-Hispanic White racial/ethnic groups.[39]

Here, we examined survival outcomes from sepsis secondary to UTIs in African American/Black and Non-Hispanic White patients (N = 166) from the ProCESS cohort[43] with the goal of understanding disparities and the molecular response to UTIs that may both be associated with sepsis survival outcomes. Because the host response to sepsis may be source-specific,[41,42] and because mortality rates differ between sepsis secondary to intra-abdominal infections and UTIs,[40] it was important to examine the question of racial disparities in another primary infection source. Survival outcomes were assessed at 90 days post-ED admission. Overall, our plasma proteomics analyses suggest that sepsis survivors, regardless of racial background, experience less inflammation and have a corresponding higher degree of endothelial barrier integrity than non-survivors. Sepsis survivors also appear to have greater anti-oxidant levels and more beneficial actin dynamics than non-survivors. Several key inflammatory and/or endothelial system proteins that support these findings were lower in sepsis survivors than non-survivors, such as: growth-regulated α protein; S100A8; S100A9; multiple collagens; TNF receptor superfamily member 1A; VCAM1; prostaglandin reductase 2 (PTGR2); MARCKS; calmodulin; multiple cadherins;

cAMP-dependent protein kinase type I-α regulatory subunit; Ras-related C3 botulinum toxin substrate 1; and nectins 2 and 3.

These changes are consistent with others' reports of sepsis survival and disease severity of UTIs via discovery and targeted LC-MS/MS, enzyme-linked immunosorbent assays, real-time reverse transcription polymerase chain reaction, and Western blotting.[54–61] Similar to our previous analysis of patients with primary intra-abdominal infection, proteins related to inflammation and hepatic fibrosis/hepatic stellate cell activation were decreased in sepsis survivors,[39] including VCAM1, which has also been proposed as a diagnostic biomarker for sepsis secondary to UTIs.[52] Therefore, these pathways and related molecules may be key factors involved in sepsis survival across primary infection sources, especially soluble forms of proteins like VCAM1. Interestingly, pathways related to the endothelial system were not significantly altered in our previous analysis of patients with primary intra-abdominal infection,[39] suggesting possible source-specific responses.

Lower levels of nectin 2 may indicate decreased UPEC invasion,[62] and several differentially-expressed proteins enriched in female reproductive and genital systems were observed. These findings suggest that inflammation and endothelial barrier integrity in the urogenital system may be important in survival outcomes related to sepsis secondary to UTIs. Tissue-specific responses based on the primary infection site may be important aspects to investigate in future studies.

Factors Associated with Survival Outcomes and Racial Background of Patients

Metabolism was not significantly impacted in Non-Hispanic White sepsis survivors but was altered in African American/Black sepsis survivors. For example, PTGR2 was differentially-expressed in African American/Black survivors compared to non-survivors, but this was not the case in Non-Hispanic White survivors. Lower levels of proteins such as PTGR2 and long-chain-fatty-acid--CoA ligase (ACSL1) suggest altered metabolism and less inflammation in African American/Black survivors compared to non-survivors.[63–65] Similar changes in ACSL1 have been demonstrated with whole genome microarrays.[64,65] Metabolism, particularly fatty acid transport and β-oxidation, were also heavily implicated in sepsis survivors in an integrated proteomics and metabolomics dataset from a cohort of mostly Black sepsis patients with primary CAP.[34] Future work should focus on these pathways as targets for improving patient survival outcomes in sepsis.

We identified 71 proteins with a significant race-survival outcome interaction term, including proteins involved in a variety of metabolic processes and the immune response, specifically in the response to the cytokine TNF and regulation of T-cell proliferation. Previously, we identified pathways related to T-cell activation and proliferation that were only significantly altered in African American/Black sepsis survivors with primary intra-abdominal infections.[39] Human leukocyte antigen class I histocompatibility antigen, A α chain protein had a significant race-survival outcome interaction term in both this and our prior study[39] and is known to have several polymorphisms across racial/ethnic backgrounds that affect the immune system.[66,67] Future studies are needed to understand T-cell activation, regulation, and/or proliferation functions in diverse racial and ethnic patient populations. Zinc finger protein 534 was also identified in this set of 71 proteins. Metal-binding genes,

such as zinc finger genes, impact the immune response[68] and previously were reported as altered between White and Black CAP patients in a transcriptomics study.[23]

Most importantly, when we compared results from two statistical approaches to understand the impact of racial/ethnic background, 16 proteins overlapped and were identified as having the most substantial relevance to racial/ethnic background and sepsis survival. These proteins were decreased in survivors from both racial groups. Interestingly, few of these proteins have previously been identified in studies of UTIs or sepsis from any primary infection source. Six of these proteins are involved in translation initiation, particularly EIF2 signaling, and are decreased in survivors at the early timepoint of hospital admission, suggesting that survivors may be experiencing greater degrees of translation shutdown as an acute response to sepsis, or prior as a result of the UTI. Although persistent shutdown is detrimental, initially, translation shutdown is beneficial toward preserved cellular energy, a regulated cellular stress response, and less cytokine production.[69,70] African American/Black sepsis non-survivors had the greatest abundances of these proteins, which may also suggest that translation is a partial contributor to these patients' survival post-ED admission. Overall, these 16 proteins provide novel changes in sepsis secondary to UTIs and because they change similarly across racial groups should be explored as prognostic biomarkers for sepsis.

Study Strengths and Limitations

While a sample size of 166 patients (45 of whom identified as African American/Black) is larger than most similar studies,[29–33,35,38] sample size could be improved in future studies, especially among African American/Black patients. Only patients with primary UTIs were selected to control for heterogeneity from multiple infection sources. All ProCESS hospitals were academic, so hospital type was not a source of variation in this study. All regression models were adjusted for age and sex because age affects the plasma proteome of sepsis patients, and both age and sex affect the risk of developing UTIs.[1,3,50]

However, information about various socioeconomic factors that could contribute to disparities, such as patients' geographical region, income, and health insurance status,[11,13,15,19,21,71–77] were not included in the original study design[43] and therefore not included as covariates. Additionally, ProCESS did not enroll healthy, non-infected patients or patients who were infected, but not septic.[43] Therefore, there were no non-septic controls in our analysis to allow for comparisons across pathophysiological conditions, representing a key limitation to this work.

CONCLUSIONS

Quantitative proteomics analysis of plasma samples from a racially and ethnically diverse cohort of sepsis survivors and non-survivors with primary UTI suggests that proteins in the immune response and endothelial system impact patient survival after ED admission. Sepsis survivors experienced less inflammation, less endothelial barrier damage, and greater anti-oxidant levels than non-survivors. These changes may be particularly relevant in the urogenital system. While several differentially-expressed proteins were identified within Non-Hispanic White (mostly related to the immune system) or African American/

Black (primarily metabolic proteins) patients, early translation shutdown was experienced universally by all sepsis survivors in this study, regardless of racial background. These studies have provided molecular insight to factors that may contribute to sepsis survival from UTIs and partially explain potential differences in outcomes for racial and ethnic groups. Larger scale studies which examine diverse patient cohorts across various primary sources of infection and other racial and ethnic groups are still warranted to better explain disparities in patient survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

The authors acknowledge funding from Vanderbilt University Start-Up Funds (RASR) and the Vanderbilt Institute of Chemical Biology (5T32GM065086 fellowship to KLK). ProCESS was supported by a grant (P50 GM076659) from the National Institute of General Medical Sciences, National Institutes of Health.

This work was supported by grants (P50GM076659, T32GM065086) from the National Institute of General Medical Sciences, National Institutes of Health.

REFERENCES

1. Medina M, Castillo-Pino E. An introduction to the epidemiology and burden of urinary tract infections. Ther Adv Urol Jan-Dec 2019;11:1756287219832172. doi:10.1177/1756287219832172 [PubMed: 31105774]
2. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol May 2015;13(5):269–84. doi:10.1038/nrmicro3432 [PubMed: 25853778]
3. Schmiemann G, Kniehl E, Gebhardt K, Matejczyk MM, Hummers-Pradier E. The diagnosis of urinary tract infection: a systematic review. Dtsch Arztebl Int May 2010;107(21):361–7. doi:10.3238/arztebl.2010.0361 [PubMed: 20539810]
4. Hooton TM. Clinical practice. Uncomplicated urinary tract infection. N Engl J Med Mar 15 2012;366(11):1028–37. doi:10.1056/NEJMcp1104429 [PubMed: 22417256]
5. Lichtenberger P, Hooton TM. Complicated urinary tract infections. Curr Infect Dis Rep Nov 2008;10(6):499–504. doi:10.1007/s11908-008-0081-0 [PubMed: 18945392]
6. Hsiao CY, Chen TH, Lee YC, Hsiao MC, Hung PH, Wang MC. Risk factors for uroseptic shock in hospitalized patients aged over 80 years with urinary tract infection. Ann Transl Med Apr 2020;8(7):477. doi:10.21037/atm.2020.03.95 [PubMed: 32395521]
7. Chou EH, Mann S, Hsu TC, et al. Incidence, trends, and outcomes of infection sites among hospitalizations of sepsis: A nationwide study. PLoS One 2020;15(1):e0227752. doi:10.1371/journal.pone.0227752 [PubMed: 31929577]
8. Jeganathan N, Yau S, Ahuja N, et al. The characteristics and impact of source of infection on sepsis-related ICU outcomes. J Crit Care Oct 2017;41:170–176. doi:10.1016/j.jcrc.2017.05.019 [PubMed: 28564621]
9. Keneally RJ, Chow JH, Pla RA, Heinz ER, Mazzeffi MA. Racial disparities in catheter related urinary tract infections among elderly trauma patients in the US. Am J Infect Control Jan 2022;50(1):77–80. doi:10.1016/j.ajic.2021.08.018 [PubMed: 34955191]
10. Bradley MS, Stanger M, Ford C, Lowder J, Handa VL. Characteristics Associated With Repeated Evaluations for Urinary Tract Infections in Older Women: A Case-Control Study. Female Pelvic Med Reconstr Surg Apr 1 2022;28(4):e133–e136. doi:10.1097/SPV.0000000000001129 [PubMed: 35234180]

11. Kempker JA, Kramer MR, Waller LA, Martin GS. Risk Factors for Septicemia Deaths and Disparities in a Longitudinal US Cohort. Open Forum Infect Dis Dec 2018;5(12):ofy305. doi:10.1093/ofid/ofy305 [PubMed: 30568980]
12. Mayr FB, Yende S, Linde-Zwirble WT, et al. Infection rate and acute organ dysfunction risk as explanations for racial differences in severe sepsis. JAMA Jun 23 2010;303(24):2495–503. doi:10.1001/jama.2010.851 [PubMed: 20571016]
13. Matter ML, Shvetsov YB, Dugay C, et al. High mortality due to sepsis in Native Hawaiians and African Americans: The Multiethnic Cohort. PLoS One 2017;12(5):e0178374. doi:10.1371/journal.pone.0178374 [PubMed: 28558016]
14. Cheek JE, Holman RC, Redd JT, Haberling D, Hennessy TW. Infectious disease mortality among American Indians and Alaska Natives, 1999–2009. Am J Public Health Jun 2014;104 Suppl 3:S446–52. doi:10.2105/AJPH.2013.301721 [PubMed: 24754622]
15. Prest J, Sathananthan M, Jeganathan N. Current Trends in Sepsis-Related Mortality in the United States. Crit Care Med Aug 1 2021;49(8):1276–1284. doi:10.1097/CCM.0000000000005017 [PubMed: 34261926]
16. Esper AM, Moss M, Lewis CA, Nisbet R, Mannino DM, Martin GS. The role of infection and comorbidity: Factors that influence disparities in sepsis. Crit Care Med Oct 2006;34(10):2576–82. doi:10.1097/01.CCM.0000239114.50519.0E [PubMed: 16915108]
17. Kramarow EA. Sepsis-related Mortality Among Adults Aged 65 and Over: United States, 2019. NCHS Data Brief Nov 2021;(422):1–8.
18. Kokofer A, Mamandipoor B, Flamm M, et al. The impact of ethnic background on ICU care and outcome in sepsis and septic shock - A retrospective multicenter analysis on 17,949 patients. BMC Infect Dis Mar 31 2023;23(1):194. doi:10.1186/s12879-023-08170-7 [PubMed: 37003970]
19. Barnato AE, Alexander SL, Linde-Zwirble WT, Angus DC. Racial variation in the incidence, care, and outcomes of severe sepsis: analysis of population, patient, and hospital characteristics. Am J Respir Crit Care Med Feb 1 2008;177(3):279–84. doi:10.1164/rccm.200703-480OC [PubMed: 17975201]
20. Jones JM, Fingar KR, Miller MA, et al. Racial Disparities in Sepsis-Related In-Hospital Mortality: Using a Broad Case Capture Method and Multivariate Controls for Clinical and Hospital Variables, 2004–2013. Crit Care Med Dec 2017;45(12):e1209–e1217. doi:10.1097/CCM.0000000000002699 [PubMed: 28906287]
21. Goodwin AJ, Nadig NR, McElligott JT, Simpson KN, Ford DW. Where You Live Matters: The Impact of Place of Residence on Severe Sepsis Incidence and Mortality. Chest Oct 2016;150(4):829–836. doi:10.1016/j.chest.2016.07.004 [PubMed: 27445093]
22. Corl K, Levy M, Phillips G, Terry K, Friedrich M, Trivedi AN. Racial And Ethnic Disparities In Care Following The New York State Sepsis Initiative. Health Aff (Millwood) Jul 2019;38(7):1119–1126. doi:10.1377/hlthaff.2018.05381 [PubMed: 31260359]
23. Peck Palmer OM, Rogers G, Yende S, Angus DC, Clermont G, Langston MA. Graph Theoretical Analysis of Genome-Scale Data: Examination of Gene Activation Occurring in the Setting of Community-Acquired Pneumonia. Shock Jul 2018;50(1):53–59. doi:10.1097/SHK.0000000000001029 [PubMed: 29049138]
24. Lutsey PL, Cushman M, Steffen LM, et al. Plasma hemostatic factors and endothelial markers in four racial/ethnic groups: the MESA study. J Thromb Haemost Dec 2006;4(12):2629–35. doi:10.1111/j.1538-7836.2006.02237.x [PubMed: 17002663]
25. Mayr FB, Spiel AO, Leitner JM, et al. Racial differences in endotoxin-induced tissue factor-triggered coagulation. J Thromb Haemost Apr 2009;7(4):634–40. doi:10.1111/j.1538-7836.2009.03307.x [PubMed: 19187081]
26. Ferguson JF, Patel PN, Shah RY, et al. Race and gender variation in response to evoked inflammation. J Transl Med Mar 12 2013;11:63. doi:10.1186/1479-5876-11-63 [PubMed: 23497455]
27. Chaudhary NS, Moore JX, Zakai NA, et al. APOL1 Nephropathy Risk Alleles and Risk of Sepsis in Blacks. Clin J Am Soc Nephrol Dec 6 2019;14(12):1733–1740. doi:10.2215/CJN.04490419 [PubMed: 31704668]

28. Wu J, Ma Z, Raman A, et al. APOL1 risk variants in individuals of African genetic ancestry drive endothelial cell defects that exacerbate sepsis. Immunity Nov 9 2021;54(11):2632–2649 e6. doi:10.1016/j.immuni.2021.10.004 [PubMed: 34715018]
29. Sharma NK, Ferreira BL, Tashima AK, et al. Lipid metabolism impairment in patients with sepsis secondary to hospital acquired pneumonia, a proteomic analysis. Clin Proteomics 2019;16:29. doi:10.1186/s12014-019-9252-2 [PubMed: 31341447]
30. Sharma NK, Tashima AK, Brunialti MKC, et al. Proteomic study revealed cellular assembly and lipid metabolism dysregulation in sepsis secondary to community-acquired pneumonia. Sci Rep Nov 15 2017;7(1):15606. doi:10.1038/s41598-017-15755-1 [PubMed: 29142235]
31. Raju MS, Jahnavi V, Kamaraju RS, et al. Continuous evaluation of changes in the serum proteome from early to late stages of sepsis caused by Klebsiella pneumoniae. Mol Med Rep Jun 2016;13(6):4835–44. doi:10.3892/mmr.2016.5112 [PubMed: 27082932]
32. Kalenka A, Feldmann RE Jr., Otero K, Maurer MH, Waschke KF, Fiedler F. Changes in the serum proteome of patients with sepsis and septic shock. Anesth Analg Dec 2006;103(6):1522–6. doi:10.1213/01.ane.0000242533.59457.70 [PubMed: 17122233]
33. Triantafilou M, Mouratis MA, Lepper PM, et al. Serum proteins modulate lipopolysaccharide and lipoteichoic acid-induced activation and contribute to the clinical outcome of sepsis. Virulence Mar-Apr 2012;3(2):136–45. doi:10.4161/viru.19077 [PubMed: 22460642]
34. Langley RJ, Tsalik EL, van Velkinburgh JC, et al. An integrated clinico-metabolomic model improves prediction of death in sepsis. Sci Transl Med Jul 24 2013;5(195):195ra95. doi:10.1126/scitranslmed.3005893
35. Seymour CW, Yende S, Scott MJ, et al. Metabolomics in pneumonia and sepsis: an analysis of the GenIMS cohort study. Intensive Care Med Aug 2013;39(8):1423–34. doi:10.1007/s00134-013-2935-7 [PubMed: 23673400]
36. Cuello F, Shankar-Hari M, Mayr U, et al. Redox state of pentraxin 3 as a novel biomarker for resolution of inflammation and survival in sepsis. Mol Cell Proteomics Oct 2014;13(10):2545–57. doi:10.1074/mcp.M114.039446 [PubMed: 24958171]
37. Punyadeera C, Schneider EM, Schaffer D, et al. A biomarker panel to discriminate between systemic inflammatory response syndrome and sepsis and sepsis severity. J Emerg Trauma Shock Jan 2010;3(1):26–35. doi:10.4103/0974-2700.58666 [PubMed: 20165718]
38. Su L, Cao L, Zhou R, et al. Identification of novel biomarkers for sepsis prognosis via urinary proteomic analysis using iTRAQ labeling and 2D-LC-MS/MS. PLoS One 2013;8(1):e54237. doi:10.1371/journal.pone.0054237 [PubMed: 23372690]
39. Kapp KL, Arul AB, Zhang KC, et al. Proteomic changes associated with racial background and sepsis survival outcomes. Mol Omics Dec 5 2022;18(10):923–937. doi:10.1039/d2mo00171c [PubMed: 36097965]
40. Leligdowicz A, Dodek PM, Norena M, et al. Association between source of infection and hospital mortality in patients who have septic shock. Am J Respir Crit Care Med May 15 2014;189(10):1204–13. doi:10.1164/rccm.201310-1875OC [PubMed: 24635548]
41. Peters-Sengers H, Butler JM, Uhel F, et al. Source-specific host response and outcomes in critically ill patients with sepsis: a prospective cohort study. Intensive Care Med Jan 2022;48(1):92–102. doi:10.1007/s00134-021-06574-0 [PubMed: 34902047]
42. Neugebauer S, Giamarellos-Bourboulis EJ, Pelekanou A, et al. Metabolite Profiles in Sepsis: Developing Prognostic Tools Based on the Type of Infection. Crit Care Med Sep 2016;44(9):1649–62. doi:10.1097/CCM.0000000000001740 [PubMed: 27097292]
43. Yealy DM, Kellum JA, Huang DT, et al. A randomized trial of protocol-based care for early septic shock. N Engl J Med. May 1 2014;370(18):1683–93. doi:10.1056/NEJMoa1401602 [PubMed: 24635773]
44. Pike F, Yealy DM, Kellum JA, et al. Protocolized Care for Early Septic Shock (ProCESS) statistical analysis plan. Crit Care Resusc Dec 2013;15(4):301–10. [PubMed: 24289512]
45. Ping L, Kundinger SR, Duong DM, et al. Global quantitative analysis of the human brain proteome and phosphoproteome in Alzheimer's disease. Sci Data Sep 28 2020;7(1):315. doi:10.1038/s41597-020-00650-8 [PubMed: 32985496]

46. Vizcaino JA, Csordas A, del-Toro N, et al. 2016 update of the PRIDE database and its related tools. Nucleic Acids Res Jan 4 2016;44(D1):D447–56. doi:10.1093/nar/gkv1145 [PubMed: 26527722]
47. Plubell DL, Wilmarth PA, Zhao Y, et al. Extended Multiplexing of Tandem Mass Tags (TMT) Labeling Reveals Age and High Fat Diet Specific Proteome Changes in Mouse Epididymal Adipose Tissue. Mol Cell Proteomics May 2017;16(5):873–890. doi:10.1074/mcp.M116.065524 [PubMed: 28325852]
48. Stepler KE, Mahoney ER, Kofler J, Hohman TJ, Lopez OL, Robinson RAS. Inclusion of African American/Black adults in a pilot brain proteomics study of Alzheimer's disease. Neurobiol Dis Dec 2020;146:105129. doi:10.1016/j.nbd.2020.105129 [PubMed: 33049317]
49. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing 2021. https://www.R-project.org/
50. Cao Z, Yende S, Kellum JA, Angus DC, Robinson RAS. Proteomics reveals age-related differences in the host immune response to sepsis. J Proteome Res Feb 7 2014;13(2):422–32. doi:10.1021/pr400814s [PubMed: 24266763]
51. Szklarczyk D, Franceschini A, Wyder S, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res Jan 2015;43(Database issue):D447–52. doi:10.1093/nar/gku1003 [PubMed: 25352553]
52. Ge G, Zheng Q, Sun Z, et al. Proteomic Signature of Urosepsis: From Discovery in a Rabbit Model to Validation in Humans. J Proteome Res Jun 30 2021;doi:10.1021/acs.jproteome.1c00189
53. Yang XK, Wang N, Yang C, Wang YM, Che TJ. Differential protein expression in patients with urosepsis. Chin J Traumatol Dec 2018;21(6):316–322. doi:10.1016/j.cjtee.2018.07.003 [PubMed: 30340979]
54. Tu H, Lai X, Li J, Huang L, Liu Y, Cao J. Interleukin-26 is overexpressed in human sepsis and contributes to inflammation, organ injury, and mortality in murine sepsis. Crit Care Aug 29 2019;23(1):290. doi:10.1186/s13054-019-2574-7 [PubMed: 31464651]
55. Zaghloul N, Addorisio ME, Silverman HA, et al. Forebrain Cholinergic Dysfunction and Systemic and Brain Inflammation in Murine Sepsis Survivors. Front Immunol 2017;8:1673. doi:10.3389/fimmu.2017.01673 [PubMed: 29326685]
56. Dubois C, Marce D, Faivre V, et al. High plasma level of S100A8/S100A9 and S100A12 at admission indicates a higher risk of death in septic shock patients. Sci Rep Oct 30 2019;9(1):15660. doi:10.1038/s41598-019-52184-8 [PubMed: 31666644]
57. Lee SM, Suk K, Lee WH. Myristoylated alanine-rich C kinase substrate (MARCKS) regulates the expression of proinflammatory cytokines in macrophages through activation of p38/JNK MAPK and NF-kappaB. Cell Immunol Aug 2015;296(2):115–21. doi:10.1016/j.cellimm.2015.04.004 [PubMed: 25929183]
58. Armbruster CE, Smith SN, Mody L, Mobley HLT. Urine Cytokine and Chemokine Levels Predict Urinary Tract Infection Severity Independent of Uropathogen, Urine Bacterial Burden, Host Genetics, and Host Age. Infect Immun Sep 2018;86(9)doi:10.1128/IAI.00327-18
59. Reyes L, Alvarez S, Allam A, Reinhard M, Brown MB. Complicated urinary tract infection is associated with uroepithelial expression of proinflammatory protein S100A8. Infect Immun Oct 2009;77(10):4265–74. doi:10.1128/IAI.00458-09 [PubMed: 19667050]
60. van Zoelen MA, Vogl T, Foell D, et al. Expression and role of myeloid-related protein-14 in clinical and experimental sepsis. Am J Respir Crit Care Med Dec 1 2009;180(11):1098–106. doi:10.1164/rccm.200810-1552OC [PubMed: 19762566]
61. Chen IJ, Hee SW, Liao CH, et al. Targeting the 15-keto-PGE2-PTGR2 axis modulates systemic inflammation and survival in experimental sepsis. Free Radic Biol Med Feb 1 2018;115:113–126. doi:10.1016/j.freeradbiomed.2017.11.016 [PubMed: 29175486]
62. Wang C, Li Q, Lv J, et al. Alpha-hemolysin of uropathogenic Escherichia coli induces GM-CSF-mediated acute kidney injury. Mucosal Immunol Jan 2020;13(1):22–33. doi:10.1038/s41385-019-0225-6 [PubMed: 31719643]
63. Mashek DG, Li LO, Coleman RA. Long-chain acyl-CoA synthetases and fatty acid channeling. Future Lipidol Aug 2007;2(4):465–476. doi:10.2217/17460875.2.4.465 [PubMed: 20354580]

64. Roelands J, Garand M, Hinchcliff E, et al. Long-Chain Acyl-CoA Synthetase 1 Role in Sepsis and Immunity: Perspectives From a Parallel Review of Public Transcriptome Datasets and of the Literature. Front Immunol 2019;10:2410. doi:10.3389/fimmu.2019.02410 [PubMed: 31681299]

65. Khaenam P, Rinchai D, Altman MC, et al. A transcriptomic reporter assay employing neutrophils to measure immunogenic activity of septic patients' plasma. J Transl Med Mar 11 2014;12:65. doi:10.1186/1479-5876-12-65 [PubMed: 24612859]

66. Cyr DD, Allen AS, Du GJ, et al. Evaluating genetic susceptibility to Staphylococcus aureus bacteremia in African Americans using admixture mapping. Genes Immun Mar 2017;18(2):95–99. doi:10.1038/gene.2017.6 [PubMed: 28332560]

67. DeLorenze GN, Nelson CL, Scott WK, et al. Polymorphisms in HLA Class II Genes Are Associated With Susceptibility to Staphylococcus aureus Infection in a White Population. J Infect Dis Mar 1 2016;213(5):816–23. doi:10.1093/infdis/jiv483 [PubMed: 26450422]

68. Rink L, Haase H. Zinc homeostasis and immunity. Trends Immunol Jan 2007;28(1):1–4. doi:10.1016/j.it.2006.11.005 [PubMed: 17126599]

69. Harding HP, Zhang Y, Scheuner D, Chen JJ, Kaufman RJ, Ron D. Ppp1r15 gene knockout reveals an essential role for translation initiation factor 2 alpha (eIF2alpha) dephosphorylation in mammalian development. Proc Natl Acad Sci U S A Feb 10 2009;106(6):1832–7. doi:10.1073/pnas.0809632106 [PubMed: 19181853]

70. Costa-Mattioli M, Walter P. The integrated stress response: From mechanism to disease. Science Apr 24 2020;368(6489)doi:10.1126/science.aat5314

71. Ogundipe F, Kodadhala V, Ogundipe T, Mehari A, Gillum R. Disparities in Sepsis Mortality by Region, Urbanization, and Race in the USA: a Multiple Cause of Death Analysis. J Racial Ethn Health Disparities Jun 2019;6(3):546–551. doi:10.1007/s40615-018-00553-w [PubMed: 30607577]

72. Wang HE, Devereaux RS, Yealy DM, Safford MM, Howard G. National variation in United States sepsis mortality: a descriptive study. Int J Health Geogr Feb 15 2010;9:9. doi:10.1186/1476-072X-9-9 [PubMed: 20156361]

73. Moore JX, Donnelly JP, Griffin R, et al. Community characteristics and regional variations in sepsis. Int J Epidemiol Oct 1 2017;46(5):1607–1617. doi:10.1093/ije/dyx099 [PubMed: 29121335]

74. Storm L, Schnegelsberg A, Mackenhauer J, Andersen LW, Jessen MK, Kirkegaard H. Socioeconomic status and risk of intensive care unit admission with sepsis. Acta Anaesthesiol Scand Aug 2018;62(7):983–992. doi:10.1111/aas.13114 [PubMed: 29569230]

75. Mayr FB, Yende S, Angus DC. Racial Disparities in Infection and Sepsis: Does Biology Matter? In: Vincent J-L, ed. Yearbook of Intensive Care and Emergency Medicine Springer-Verlag Berlin Heidelburg; 2008:24–30.

76. Dombrovskiy VY, Martin AA, Sunderram J, Paz HL. Occurrence and outcomes of sepsis: influence of race. Crit Care Med Mar 2007;35(3):763–8. doi:10.1097/01.CCM.0000256726.80998.BF [PubMed: 17255870]

77. Baghdadi JD, Wong M, Comulada WS, Uslan DZ. Lack of insurance as a barrier to care in sepsis: A retrospective cohort study. J Crit Care Aug 2018;46:134–138. doi:10.1016/j.jcrc.2018.02.005 [PubMed: 29929704]

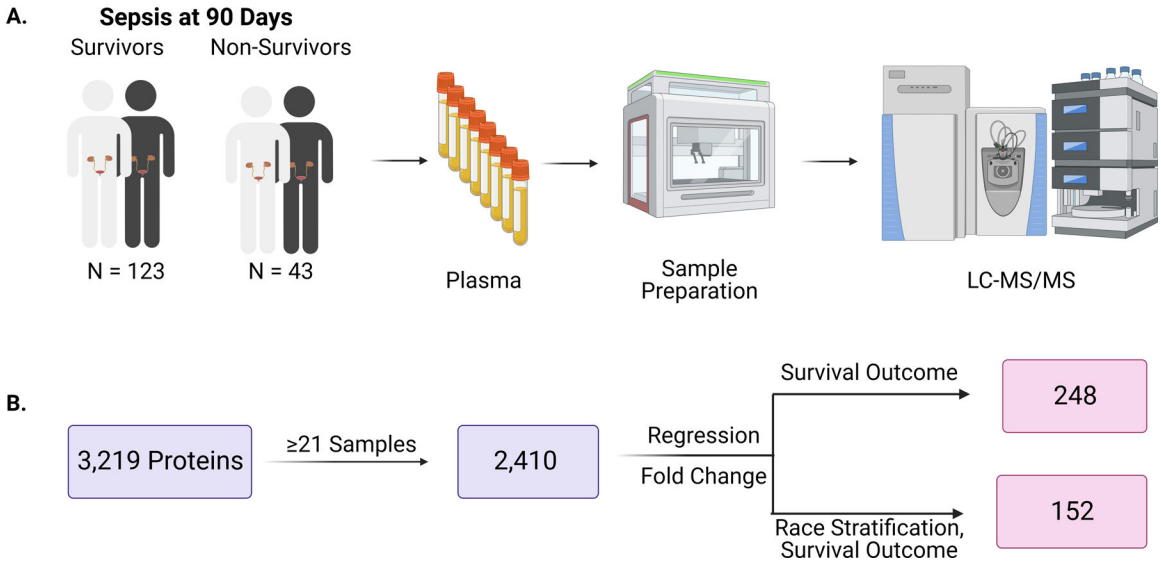

Figure 1.

General **(A)** sample preparation and **(B)** data analysis workflow. Plasma samples from sepsis patients with primary urinary tract infection were obtained at hospital admission with survival outcome referring to a patient's status 90 days post-admission. Sample preparation included tryptic digestion and TMTpro labeling and was partially automated using a Biomek i7 workstation. Fractionated TMTpro batches were analyzed via LC-MS/MS. Normalized TMTpro reporter ion intensities for proteins were analyzed using linear regression models with and without stratification by patients' racial/ethnic backgrounds (see Materials and Methods). Differentially-expressed proteins are considered as proteins with an FDR-adjusted $p < 0.05$ and significant fold change values (see Materials and Methods). Figure was created with Biorender.com. Abbreviation: LC-MS/MS = liquid chromatography-tandem mass spectrometry.

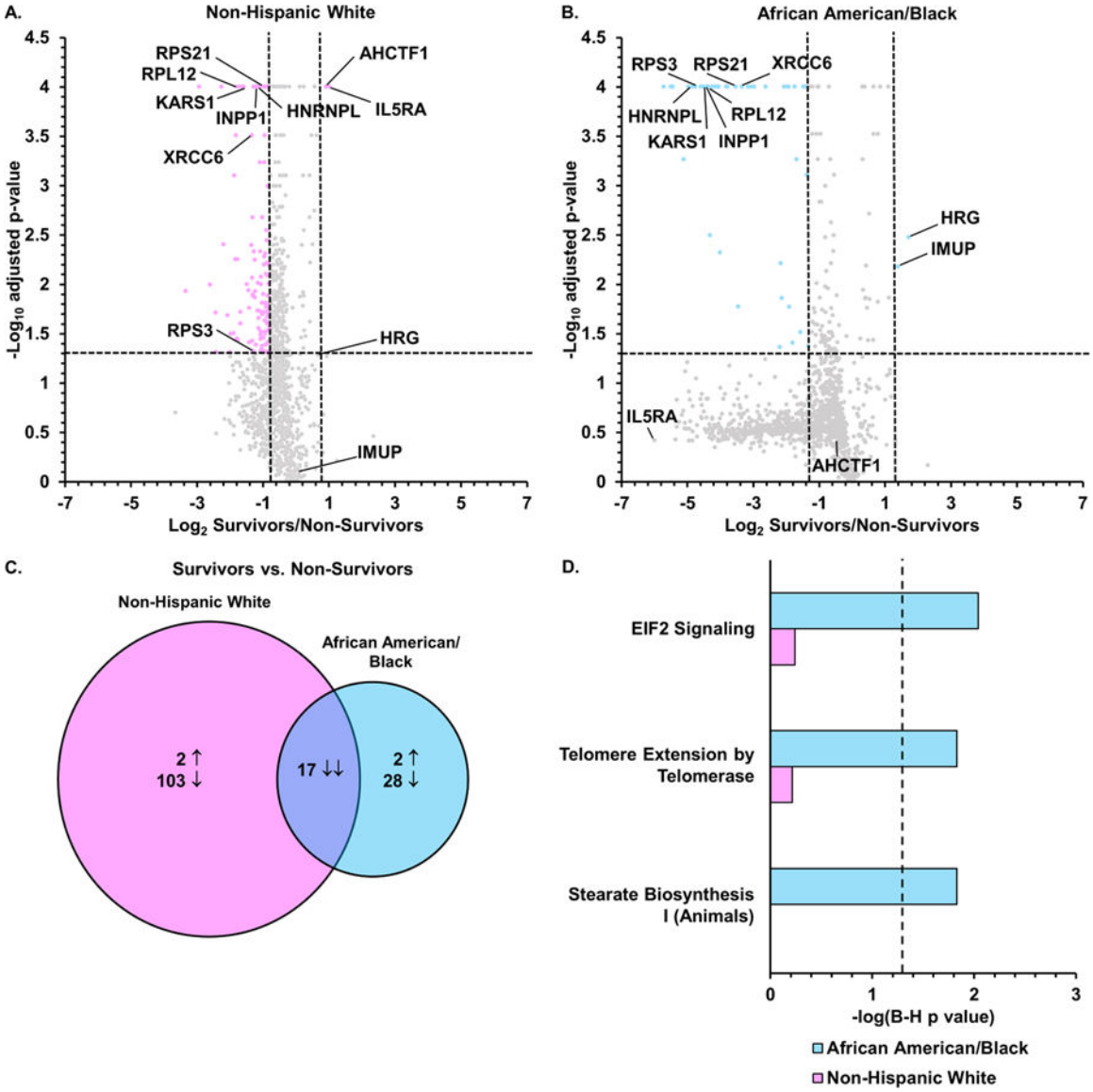

Figure 2.

Analysis of data stratified by patients' racial/ethnic backgrounds. Volcano plot of protein $\log_2$ of averaged TMTpro reporter ion abundances' ratio of sepsis survivors to non-survivors as a function of FDR-adjusted p-value (N = 1,216 proteins with un-adjusted $p < 0.05$) in **(A)** Non-Hispanic White and **(B)** African American/Black patients. Light grey data points indicate proteins with non-significant FDR-adjusted p-values (adjusted $p \geq 0.05$) and/or non-significant fold change values in Non-Hispanic White patients and African American/ Black patients. Dashed lines indicate p-value and fold change cutoffs. Example proteins are labeled. **(A)** Pink data points indicate proteins with both significant FDR-adjusted p-values (adjusted $p < 0.05$) and fold change values in Non-Hispanic White patients (N = 122 proteins). **(B)** Blue data points indicate proteins with both significant FDR-adjusted p-values (adjusted $p < 0.05$) and fold change values in African American/Black patients (N = 47 proteins). **(C)** Venn Diagram of the overlap of differentially-expressed proteins [pink and

blue proteins from **(A)** and **(B)**] between survivors and non-survivors in Non-Hispanic White and African American/Black patients. **(D)** Ingenuity Pathway Analysis of the proteins in (C) (i.e., N = 122 differentially-expressed proteins in Non-Hispanic White sepsis patients and N = 47 differentially-expressed proteins in African American/Black sepsis patients). Canonical pathways were identified from the IPA library using Fisher's exact test adjusted for multiple hypothesis testing using the Benjamini-Hochberg (B-H) correction. Abbreviations: RPS21 = 40S ribosomal protein S21; RPS3 = 40S ribosomal protein S3; RPL12 = 60S ribosomal protein L12; HNRNPL = heterogeneous nuclear ribonucleoprotein L; KARS1 = lysine-tRNA ligase; XRCC6 = X-ray repair cross-complementing protein 6; INPP1 = inositol polyphosphate 1-phosphatase; IL5RA = interleukin-5 receptor subunit α; AHCTF1 = protein ELYS; IMUP = immortalization up-regulated protein; HRG = histidine-rich glycoprotein; B-H = Benjamini-Hochberg; EIF2 = eukaryotic translation initiation factor 2.

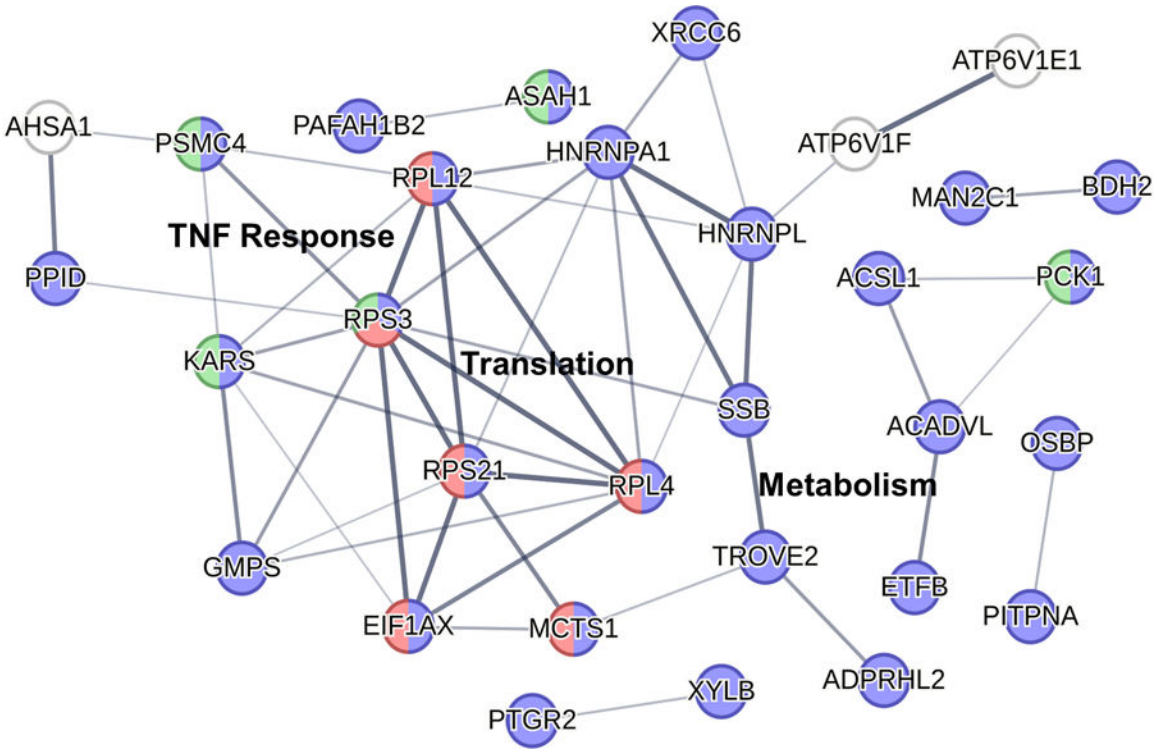

Figure 3.

STRING network of proteins with a significant race-survival outcome interaction term (N = 71). Disconnected nodes are hidden. Node color represents key functions, and line thickness indicates the strength of protein interactions. Blue represents proteins with metabolic functions, red represents proteins involved in translation, and green represents proteins involved in the tumor necrosis factor (TNF) response.

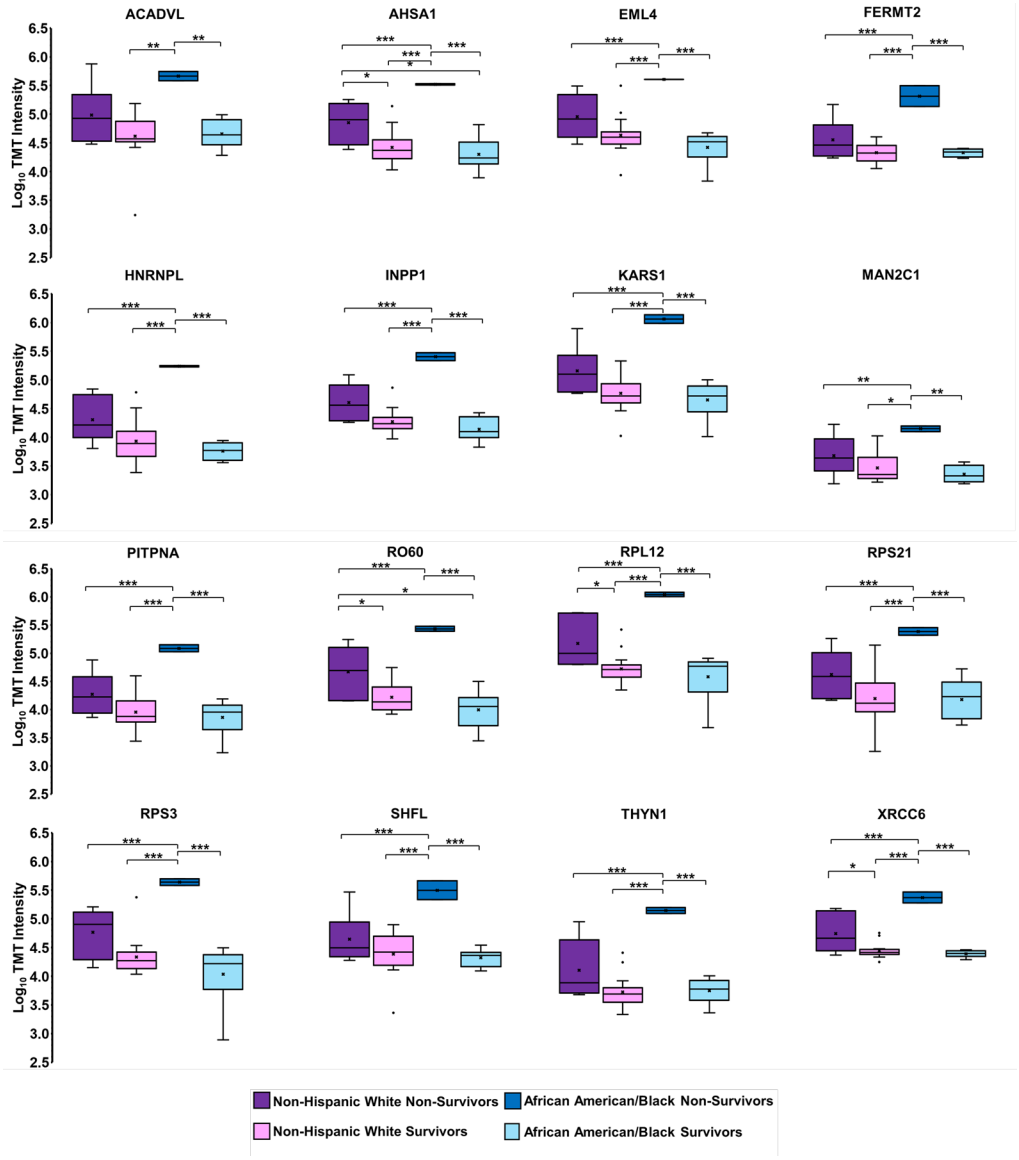

Figure 4.

Box plots of $\log_{10}$ TMTpro intensities of the 16 proteins that change in both Non-Hispanic White and African American/Black survivors are shown. The horizontal line in the box represents the median and X represents the mean. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, as determined by two-way ANOVAs with replication and Tukey-Kramer post-hoc tests. Purple represents Non-Hispanic White non-survivors, pink represents Non-Hispanic White survivors, dark blue represents African American/Black non-survivors, and light blue represents African American/Black survivors. Abbreviations: THYN1 = thymocyte nuclear protein 1; ACADVL = very long-chain specific acyl-CoA dehydrogenase, mitochondrial; 60S ribosomal protein L12; RO60 = 60 kDa SS-A/Ro ribonucleoprotein; KARS1 = Lysine-tRNA ligase; AHSA1 = activator of 90 kDa heat shock protein ATPase homolog 1; RPS3 = 40S ribosomal protein S3; INPP1 = inositol polyphosphate 1-phosphatase; PITPNA = phosphatidylinositol transfer protein α isoform; HNRNPL = heterogeneous nuclear

ribonucleoprotein L; EML4 = echinoderm microtubule-associated protein-like 4; RPS21 = 40S ribosomal protein S21; MAN2C1 = α-mannosidase 2C1; SHFL = shiftless antiviral inhibitor of ribosomal frameshifting protein; XRCC6 = X-ray repair cross-complementing protein 6; FERMT2 = fermitin family homolog 2.

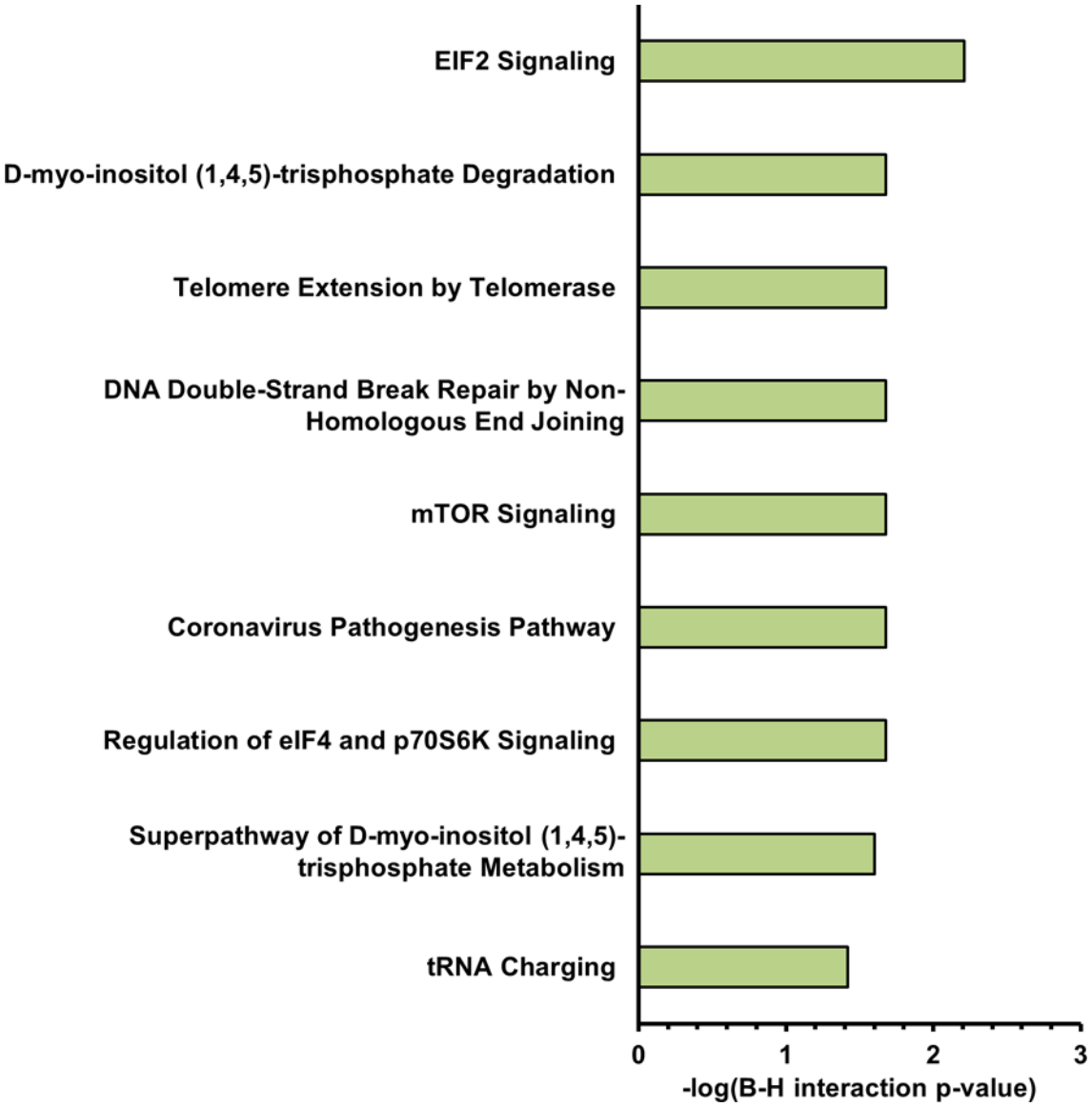

Figure 5.

Ingenuity Pathway Analysis of the differentially-expressed proteins (N = 16) in both Non-Hispanic White and African American/Black sepsis survivors that also have a significant race-survival outcome interaction. Canonical pathways were identified from the IPA library using Fisher's exact test adjusted for multiple hypothesis testing using the Benjamini-Hochberg correction. Abbreviations: B-H = Benjamini-Hochberg; EIF2 = eukaryotic translation initiation factor 2; DNA = deoxyribonucleic acid; mTOR = mammalian target of rapamycin; eIF4 = eukaryotic translation initiation factor 4F; p70S6K =P70 S6 kinase; tRNA = transfer ribonucleic acid.

Table 1.

Demographics of the UTI ProCESS Patients Selected for this Study.

Characteristic	*Survivors*		*Non-Survivors*		Statistic
	African American/ Black	**Non-Hispanic White**	**African American/ Black**	**Non-Hispanic White**	
N	35	88	10	33	
Age (years) [a]	62 ± 18	62 ± 17	68 ± 14	73 ± 14	**0.012,** 0.470, 0.453
Female (N, %)[b]	12 (34%)	56 (64%)	8 (80%)	25 (76%)	**15.271**
Renal Failure (N, %)[b]	4 (11%)	13 (15%)	2 (20%)	6 (18%)	-
History of Dialysis (N, %)[b]	0 (0%)	5 (6%)	0 (0%)	1 (3%)	-
Diabetes (N, %)[b]	13 (37%)	31 (35%)	3 (30%)	15 (45%)	1.328

[a] Average ± 1 standard deviation. P-values were calculated using a 2-way ANOVA with interaction and are for survival outcome, race, and the race-survival outcome interaction, respectively.

[b] Number of patients and percentage of total within group. χ^2 statistics were calculated using contingency table analysis. – indicates where frequencies were too small to calculate a χ^2 statistic. **Bold** denotes significant differences between groups ($p < 0.05$ or $\chi^2 > 7.815$).

Table 2.

Proteins with a Significant Race-Survival Outcome Interaction.

					Fold Change Values [b]	
	Accession Number	**Gene**	**Protein Name**	**p-value**[a]	**Non-Hispanic White**	**African American/ Black**
	P49748	**ACADVL**	**Very long-chain specific acyl-CoA dehydrogenase, mitochondrial**	**<0.001**	**0.29**	**0.11**
§	P33121	ACSL1	Long-chain-fatty-acid--CoA ligase 1	<0.001	0.60	0.02
§	Q13444	ADAM15	Disintegrin and metalloproteinase domain-containing protein 15	<0.001	0.84	0.36
	Q9NX46	ADPRS	ADP-ribose glycohydrolase ARH3	0.023	0.55	0.11
*	Q8WYP5	AHCTF1	Protein ELYS	<0.001	1.89	0.80
	O95433	**AHSA1**	**Activator of 90 kDa heat shock protein ATPase homolog 1**	**<0.001**	**0.36**	**0.07**
	O95841	ANGPTL1	Angiopoietin-related protein 1	<0.001	1.01	0.49
	Q92619	ARHGAP45	Rho GTPase-activating protein 45	<0.001	0.82	1.76
	Q92747	ARPC1A	Actin-related protein 2/3 complex subunit 1A	0.047	0.68	0.14
	Q13510	ASAH1	Acid ceramidase	0.001	0.89	0.51
§	P36543	ATP6V1E1	V-type proton ATPase subunit E 1	<0.001	1.07	0.22
	Q16864	ATP6V1F	V-type proton ATPase subunit F	0.024	0.84	0.31
§	Q9BUT1	BDH2	3-hydroxybutyrate dehydrogenase type 2	<0.001	0.67	0.03
	P07357	C8A	Complement component C8 α chain	0.033	1.00	1.34
	Q03591	CFHR1	Complement factor H-related protein 1	0.014	0.86	1.44
	O43529	CHST10	Carbohydrate sulfotransferase 10	0.002	0.64	1.50
	Q6V1P9	DCHS2	Protocadherin-23	<0.001	0.41	4.94
§	Q96HY6	DDRGK1	DDRGK domain-containing protein 1	<0.001	0.70	0.05
	Q86TI2	DPP9	Dipeptidyl peptidase 9	0.043	0.56	0.09
	P47813	EIF1AX	Eukaryotic translation initiation factor 1A, X-chromosomal	<0.001	0.81	2.15
	Q9HC35	**EML4**	**Echinoderm microtubule-associated protein-like 4**	**<0.001**	**0.47**	**0.07**
	Q15303	ERBB4	Receptor tyrosine-protein kinase erbB-4	0.001	0.99	0.41
§	P38117	ETFB	Electron transfer flavoprotein subunit β	<0.001	0.34	0.05
	Q96AC1	**FERMT2**	**Fermitin family homolog 2**	**0.018**	**0.46**	**0.09**
	P49915	GMPS	GMP synthase [glutamine-hydrolyzing]	0.048	1.01	0.23
§	P42357	HAL	Histidine ammonia-lyase	<0.001	0.36	0.06
§	P04439–2	HLA-A	Isoform 2 of HLA class I histocompatibility antigen, A α chain	<0.001	0.67	0.12
§	P09651	HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1	<0.001	0.59	0.26
	P14866	**HNRNPL**	**Heterogeneous nuclear ribonucleoprotein L**	**<0.001**	**0.44**	**0.04**
§	Q9GZP8	IMUP	Immortalization up-regulated protein	0.001	1.11	2.63
	P49441	**INPP1**	**Inositol polyphosphate 1-phosphatase**	**<0.001**	**0.41**	**0.06**
	Q15046	**KARS1**	**Lysine--tRNA ligase**	**<0.001**	**0.33**	**0.05**
	O60268	KIAA0513	Uncharacterized protein KIAA0513	0.002	1.04	2.21

	Accession Number	Gene	Protein Name	p-value[a]	Fold Change Values [b] Non-Hispanic White	Fold Change Values [b] African American/ Black
*	Q6ISS4	LAIR2	Leukocyte-associated immunoglobulin-like receptor 2	<0.001	0.32	0.99
§	P56470	LGALS4	Galectin-4	<0.001	0.31	0.03
	Q96L50	LRR1	Leucine-rich repeat protein 1	0.033	1.18	2.23
*	Q32MZ4	LRRFIP1	Leucine-rich repeat flightless-interacting protein 1	<0.001	0.52	1.02
	Q9NTJ4	**MAN2C1**	**α-mannosidase 2C1**	**<0.001**	**0.54**	**0.16**
	Q9ULC4	MCTS1	Malignant T-cell-amplified sequence 1	<0.001	0.65	2.02
	Q10469	MGAT2	α-1,6-mannosyl-glycoprotein 2-β-N-acetylglucosaminyltransferase	0.005	0.86	0.53
*	Q9Y2G1	MYRF	Myelin regulatory factor	0.012	0.42	0.96
	P22059	OSBP	Oxysterol-binding protein 1	0.021	0.60	0.11
§	Q96BN8	OTULIN	Ubiquitin thioesterase otulin	<0.001	0.71	0.30
§	P68402	PAFAH1B2	Platelet-activating factor acetylhydrolase IB subunit α2	<0.001	0.63	0.24
§	P35558	PCK1	Phosphoenolpyruvate carboxykinase, cytosolic [GTP]	<0.001	0.25	0.02
	O00625	PIR	Pirin	0.020	0.39	0.04
	Q00169	**PITPNA**	**Phosphatidylinositol transfer protein α isoform**	**<0.001**	**0.43**	**0.07**
§	Q9Y263	PLAA	Phospholipase A-2-activating protein	<0.001	0.75	0.26
§	Q8NHP8	PLBD2	Putative phospholipase B-like 2	<0.001	0.91	0.37
*	Q15063–6	POSTN	Isoform 6 of Periostin	<0.001	0.55	1.05
*	Q15063	POSTN	Periostin	0.049	0.49	1.01
	Q08752	PPID	Peptidyl-prolyl cis-trans isomerase D	<0.001	0.88	1.25
	P43686	PSMC4	26S proteasome regulatory subunit 6B	0.002	0.65	0.26
§	Q8N8N7	PTGR2	Prostaglandin reductase 2	<0.001	0.27	0.04
	P10155	**RO60**	**60 kDa SS-A/Ro ribonucleoprotein**	**<0.001**	**0.30**	**0.05**
	P30050	**RPL12**	**60S ribosomal protein L12**	**<0.001**	**0.30**	**0.05**
§	P36578	RPL4	60S ribosomal protein L4	<0.001	0.80	0.13
	P63220	**RPS21**	**40S ribosomal protein S21**	**<0.001**	**0.51**	**0.09**
	P23396	**RPS3**	**40S ribosomal protein S3**	**<0.001**	**0.41**	**0.04**
	Q9NUL5	**SHFL**	**Shiftless antiviral inhibitor of ribosomal frameshifting protein**	**0.001**	**0.40**	**0.06**
	A0MZ66	SHTN1	Shootin-1	0.001	0.43	0.14
§	P05455	SSB	Lupus La protein	<0.001	0.37	0.02
	Q9Y365	STARD10	START domain-containing protein 10	0.049	1.04	0.05
	P43405	SYK	Tyrosine-protein kinase SYK	0.017	0.92	2.17
	P37173	TGFBR2	TGF-β receptor type-2	<0.001	0.63	0.49
	Q9P016	**THYN1**	**Thymocyte nuclear protein 1**	**<0.001**	**0.28**	**0.04**
	P32971	TNFSF8	Tumor necrosis factor ligand superfamily member 8	0.002	0.87	0.50
*	P45379	TNNT2	Troponin T, cardiac muscle	<0.001	0.21	1.18
	P12956	**XRCC6**	**X-ray repair cross-complementing protein 6**	**0.003**	**0.40**	**0.10**
§	O75191	XYLB	Xylulose kinase	0.042	0.40	0.06

Accession Number	Gene	Protein Name	p-value[a]	*Fold Change Values* [b] Non-Hispanic White	African American/ Black
Q76KX8	ZNF534	Zinc finger protein 534	0.049	1.50	1.70

[a] p-values were calculated using a linear regression model and adjusted using FDR.

[b] Fold change values refer to comparisons of survivors/non-survivors.

Bold denotes proteins that are also differentially-expressed (have within-group FDR-adjusted $p < 0.05$ and meet fold change cutoff) in both Non-Hispanic White and African American/Black survivors.

* indicates proteins that are differentially-expressed in Non-Hispanic White survivors only.

§ indicates proteins that are differentially-expressed in African American/Black survivors only.