BRIEF REPORT

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Bioenergetic Crisis in ICU-Acquired Weakness Gene Signatures Was Associated With Sepsis-Related Mortality: A Brief Report

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OBJECTIVES: To investigate the relationship between ICU-acquired weakness (ICUAW) signatures and sepsis-related mortality using gene expression from the blood within 24 hours of sepsis onset.

DESIGN: Observational study using differential gene expression analysis.

SETTING: Publicly available gene expression profile GSE54514, single-center medical and surgical ICU.

PATIENTS: Patients with primary bacteremia- and respiratory-triggered sepsis including 8 nonsurvivors and 13 survivors who were 18 years old and older and admitted to ICU.

MEASUREMENTS AND MAIN RESULTS: Among validated 526 ICUAW gene signatures, differential gene expression analysis controlling for age identified 38 significantly expressed genes between nonsurvivors and survivors. Functional enrichment analysis of differentially expressed ICUAW genes identified impaired cadherin binding, sarcomere formation, and energy metabolism among nonsurvivors.

CONCLUSIONS: Our findings demonstrated a biological association between sepsis-related mortality and ICUAW signatures in the early phase of sepsis. Defects in energy metabolism and muscle fiber formation were associated with sepsis-related mortality.

KEY WORDS: intensive care unit-acquired weakness; mortality; persistent inflammation, immunosuppression, and catabolism syndrome; post-intensive care syndrome; sepsis

Sepsis is a life-threatening syndrome and a major public health concern (1). During sepsis, an infectious agent triggers a vicious cycle of pathogen-associated molecular patterns through pattern recognition receptors and damage-associated molecular patterns (2), contributing to organ failure. Mounting evidence shows mitochondrial dysfunction plays a key role in organ failure (3–8).

ICU-acquired weakness (ICUAW) is a syndrome of limb muscle weakness relating to sepsis (9, 10). ICUAW gene expression signatures were discovered from muscle biopsies of adult septic patients with ICUAW, presenting a global energy failure and mitochondrial incapability (11, 12). This raises a question as to whether biological mechanisms of sepsis-related mortality and ICUAW are overlapping.

In this work, the primary objective was to investigate whether the preselected ICUAW genes were differentially expressed between nonsurvivors and survivors within 24 hours of sepsis onset. Our secondary aim was to explore novel mechanistic pathways relating to mortality.

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KEY POINTS

Question: Identify a pathophysiologic association between sepsis-related in-hospital mortality and ICU-acquired weakness (ICUAW) gene signatures.

Findings: Differential gene expression analysis comparing nonsurvivors due to sepsis and survivors using ICUAW gene signatures identified 38 genes that were significantly expressed. Disrupted mitochondrial respiration, glycolytic metabolism, and sarcomere formation were associated with sepsis-related mortality.

Meaning: Energy derangement and muscle fiber malformation explained by acute ICUAW gene expressions may play a role in sepsis-related mortality.

MATERIALS AND METHODS

Study Design and Population

This study is a hypothesis-generating secondary analysis of publicly available Gene Expression Omnibus microarray data, GSE54514, consisting of whole blood samples from septic adults compared with healthy controls (13). In this dataset, sepsis was defined by Sepsis-2 criteria (14). This dataset consists of patients with primary bacteremia-, respiratory-, urinary tract-triggered sepsis, and unavailable site of infection described as "others" in the original study.

We excluded patients with "others" class because of nonexistent cases related to mortality. For urinary tract–triggered sepsis, there is an unbalanced number of nonsurvivors and survivors. To avoid the baseline selection bias due to the urinary tract–triggered sepsis, we included primary bacteremia- and respiratory-triggered sepsis in the primary analysis. Septic patients included in this analysis are shown in **Supplemental Figure S1** (http://links.lww.com/CCX/B102).

In the dataset, longitudinal data were available from the first day to 5 days after sepsis onset. To observe gene expression and its pathway in the acute phase of sepsis, we used day 1 data in this analysis.

Due to the use of a public and deidentified dataset, this study was deemed nonhuman subjects research and did not undergo institutional review. This study was conducted in accordance with the Declaration of Helsinki.

Microarray Data

The GSE54514 dataset was normalized and log-transformed, and no batch effect was confirmed. We excluded genes expressed in fewer than three samples, genes with an expression less than the median level gene expression for all genes in the dataset, and genes without an Entrez Gene ID (13). For Entrez Gene ID with multiple probes, we calculated the median expression value of all corresponding probes to determine the expression value for each gene. A total of 13,972 genes were available in this analysis.

ICUAW-Associated Genes

Walsh et al (11) identified 695 genes from 14 sepsis patients with mechanical ventilation for at least a week and who were diagnosed as ICUAW post ICU discharge and eight healthy controls. Among 695 ICUAW-associated genes, we excluded genes that were unavailable or duplicated by their Entrez Gene ID, with 526 remaining for analysis.

Differential Gene Expression Analysis

Age-adjusted differential gene expression analysis among ICUAW-associated genes was performed in the sepsis microarray dataset between nonsurvivors and survivors, using the R limma package with a Benjamini-Hochberg (BH) correction method and adjusted p value of less than 0.05 (15). A study workflow is shown in **Supplemental Figure S2** (http://links.lww.com/CCX/B102). In addition, a biologically significant threshold was set to an absolute value of log2(fold change) greater than 0.5. Significantly overrepresented genes were clustered by hierarchical clustering in R pheatmap package (Version 1.0.12).

Gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed to identify overrepresented GO categories and KEGG pathways for differentially expressed ICUAW-associated genes in septic patients between nonsurvivors and survivors. We used the R clusterProfiler package with BH correction for GO analysis to identify biological process,

cellular component, and molecular function and to perform KEGG pathway enrichment analysis of the differentially expressed genes using an adjusted *p* value of less than 0.05 (16). We anticipated a confounding effect on the association between ICUAW-associated genes and sepsis-related mortality due to the site of infection. Because of the limited sample size, our work of stratified analysis based on site of infection is documented in the **Supplemental Table S3** and **Figures S4-S6** (http://links.lww.com/CCX/B102). Data acquisition and statistical analyses were performed using R (Version: 4.0.1).

RESULTS

Subject Characteristics

Of 21 septic patients with the microarray data, 13 (61.9%) were survivors and eight (38.1%) were nonsurvivors. The median and the interquartile range (IQR) of age among survivors were 60.0 years (IQR, 52.0–68.0 yr) and 69.0 years (IQR, 61.5–80.0 yr) among nonsurvivors. Characteristics and demographics of the study population are shown in **Supplemental Table S1** (http://links.lww.com/CCX/B102).

Differential Gene Expression Analysis

Of the 526 genes available for analysis, a total of 38 genes were differentially expressed between nonsurvivors and survivors within 24 hours after sepsis onset (**Supplemental Fig. S3**, http://links.lww.com/CCX/B102). These 38 differentially expressed ICUAW-associated genes were clustered into nonsurvivors and survivors in the heatmap shown in **Figure 1**.

Functional Enrichment With GO and KEGG Pathway Enrichment Analysis

Functional enrichment analysis identified 17 GO terms as shown in **Figure 2**. Significantly up-regulated genes enriched membrane component pathways. Down-regulated genes enriched cadherin binding, sarcomere formation, and energy metabolism including nicotinamide adenine dinucleotide reduced form (NADH) and glycolysis. KEGG pathway enrichment analysis identified three pathways involved in the biosynthesis of amino acids, hypoxia-inducible factor (HIF)–1 signaling, and carbon metabolism with down-regulated genes (**Supplemental Table S2**, http://links.lww.com/CCX/B102).

DISCUSSION

We used preselected ICUAW-associated genes from an earlier experiment to observe an association between sepsis-related mortality and ICUAW signatures from the blood within 24 hours of sepsis onset. We observed 38 ICUAW genes that were differentially expressed between nonsurvivors and survivors. Functional enrichment analysis of these differentially expressed ICUAW-associated genes identified a failure in sarcomere formation and bioenergy production.

In sepsis, energy metabolism switches from oxidative phosphorylation to glycolytic metabolism, known as the Warburg effect (17). Energy failure in oxidative phosphorylation is associated with sepsis-related mortality (12). For ICUAW, although acute differential gene expression within 24 hours after sepsis onset has not been studied, expression profiling of mitochondrial components has shown that changes in bioenergy failure profiling from day 1 to 7 in critically ill patients were associated with muscle mass reduction (18). When compared with healthy controls, mitochondria and bioenergy metabolism were down-regulated in patients with sepsis who were diagnosed with ICUAW after ICU discharge (11). This bioenergy failure is explained by prolonged inflammation, known as persistent inflammation, immunosuppression, and catabolic syndrome (19). In our study, down-regulated ICUAW-associated genes enriched the NADH regeneration. This suggests an energy failure, specifically impaired mitochondrial respiration in ICUAW, may be present in septic patients who are nonsurvivors.

Although glycolytic energy generation is a counterpart to aerobic respiration, the rate of glycolysis did not significantly differ between chronic critical ill patients with ICUAW and healthy controls (20). Although few studies reported disrupted glycolysis in ICUAW, broad defects in energy metabolism, characterized by impaired oxidative phosphorylation and glycolysis, underlie sepsis-induced immunometabolic paralysis (21). In the immunometabolic paralysis phase of sepsis, both HIF-1 signaling and glycolytic metabolism are down-regulated, and energy metabolism shifts toward fatty acid oxidation (22). Our analysis showed

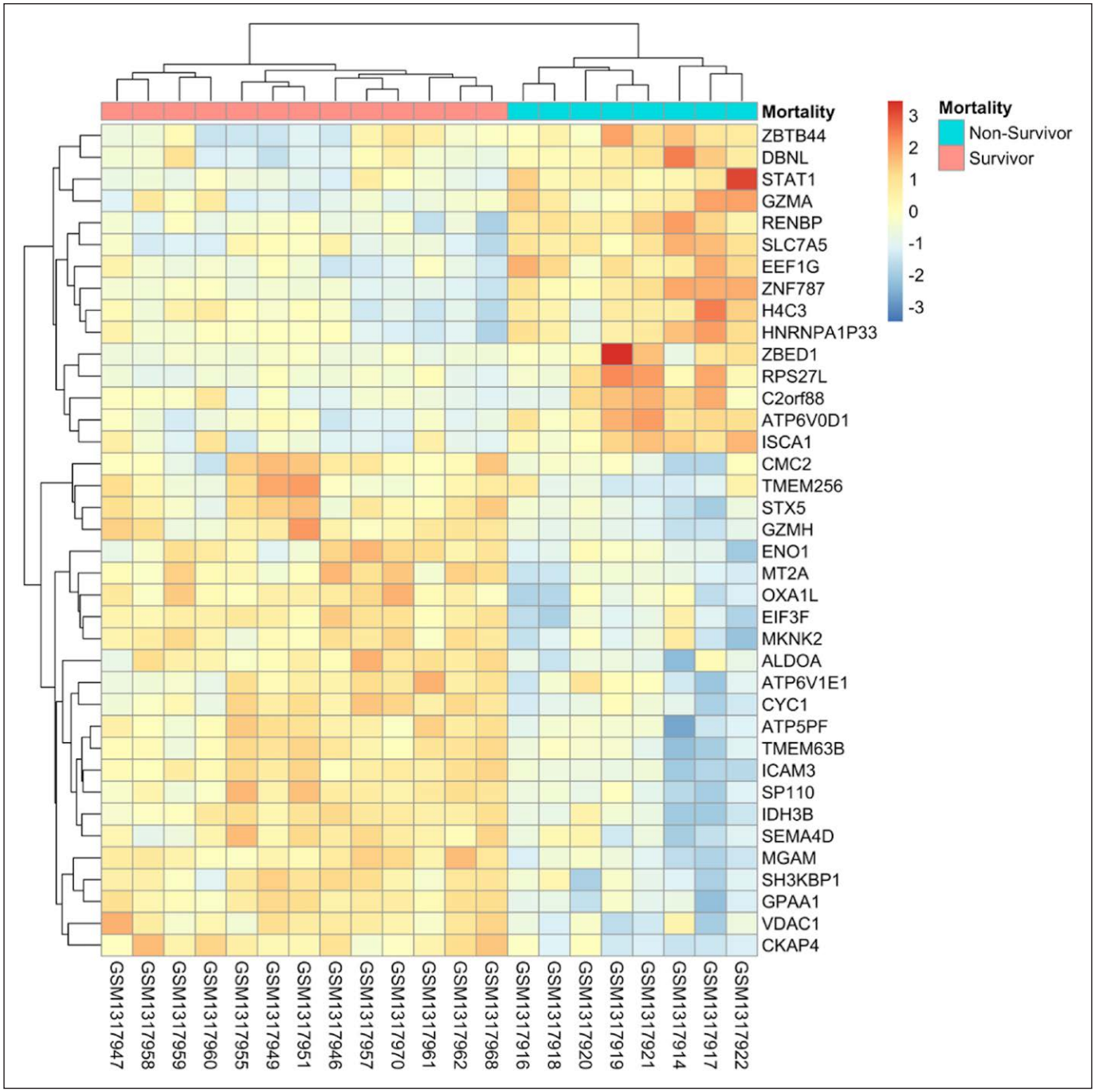

Figure 1. Heat map of 38 ICU-acquired weakness (ICUAW) differentially expressed genes (DEGs) with mortality as an annotation. ICUAW DEGs have differentiated mortality among patients with sepsis.

a down-regulation of glycolytic process and HIF-1 signaling pathway in septic nonsurvivors, suggesting those nonsurvivors may have both impaired oxidative phosphorylation and glycolysis in blood within 24 hours of sepsis onset.

Our study has several limitations. First, due to the limited sample size and available population characteristics such as Sepsis-3 definition rather than Sepsis-2 definition and medical or surgical sepsis, unmeasured confounders may produce biased genes between nonsurvivors and survivors. Second, although we discovered gene signatures of ICUAW that were differentially expressed between nonsurvivors and survivors of sepsis, we are unable to determine whether these findings may be a factor of other contributing pathophysiologic attributes that derive disease severity and mortality within this study. Third, ICUAW genes were identified by muscle

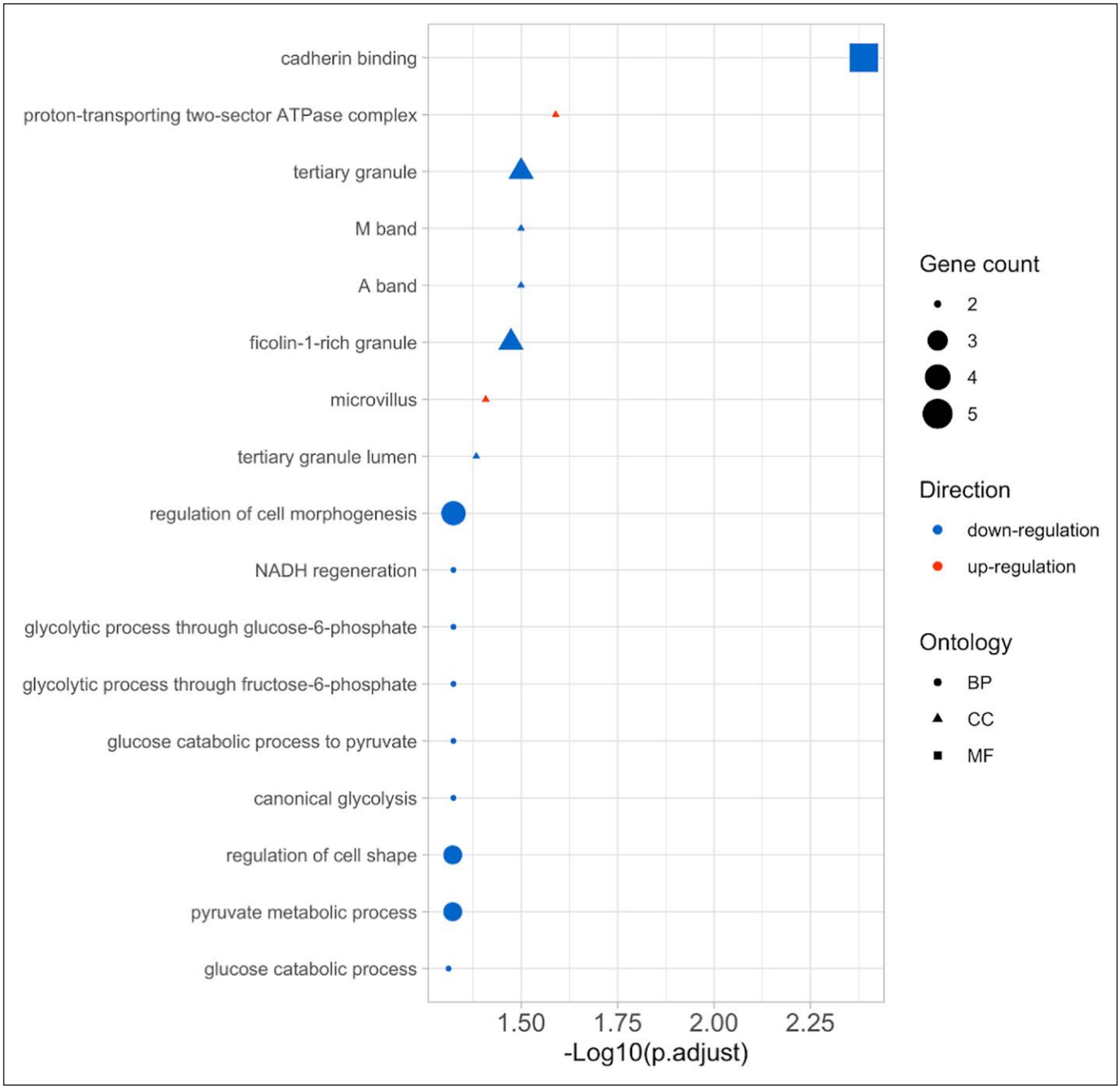

Figure 2. Gene ontology (GO) analysis of 38 ICU-acquired weakness (ICUAW) differentially expressed genes (DEGs). GO terms include the biological process (BP), cell component (CC), and molecular function (MF). Up-regulated ICUAW DEGs enriched membrane component pathways. Downregulated ICUAW DEGs enriched cadherin binding, sarcomere formation (A and M band), and energy metabolism. ATPase = adenosine triphosphatase, NADH = nicotinamide adenine dinucleotide reduced form.

biopsy, which is impractical and not performed as part of routine clinical care. Therefore, our analysis was conducted using whole blood samples; however, we acknowledge that tissue-specificity may show biased estimates of significant genes. Last, because of the limited available dataset, we were unable to implement a validation study that aim to determine the reproducibility of our results. Further exploration is necessary to determine the involvement of ICUAW genes in sepsis-related mortality in an acute phase of sepsis.

CONCLUSIONS

We identified a pathophysiologic association between ICUAW genes and sepsis-related mortality from the

blood within 24 hours of the sepsis onset. Exploration of these ICUAW-associated genes identified derangement in metabolic energy-producing pathways and sarcomere formation. Further investigation of the biological link between sepsis-related mortality and ICUAW across site of infection is warranted.

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Drs. Kobara and Kamaleswaran conceptualized a design, acquired data, performed formal analysis, and wrote an original draft. Dr. Rad acquired data and reviewed the workflow of the analysis. Drs. Grunwell and Coopersmith reviewed and supervised interpretations of the results and draft. All authors read and approved the final article.

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R scripts used for data preprocessing and analysis are available from https://github.com/seibikobara/ICUAW_DEGs. Datasets generated and analyzed during the study are available upon request.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. For the remaining authors, none were declared. All authors declare no potential conflict of interest.

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