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LONGITUDINAL ANALYSIS OF TRANSCRIPTOMIC SUBTYPES IN TRAUMA PATIENTS

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ABSTRACT—**Objective:** We previously identified two transcriptomic subtypes (Signature Groups: SG1 vs. SG2) in trauma patients at 12 hours postinjury, with SG1 associated with worse outcomes. In this study, we aimed to further characterize the changes in SG subtype categorization of trauma patients over time after injury and define the corresponding association with outcomes based on the timing of the subtype designation. **Methods and Results:** This study was a secondary analysis of published data of whole-blood leukocyte transcriptomics, a longitudinal data from 167 severe blunt trauma patients. We assigned trauma patients to SG1 or SG2 subtype for time points between 12 hours and 28 days, inclusive, postinjury and characterized their longitudinal outcomes. SG1 assignment, regardless of time point, was associated consistently with slower recovery. Further analysis revealed that additional prognostic information could be obtained by assessing SG subtype at both 12 hours and 1 day. **Conclusions:** This study provides a proof of concept that immune status can worsen after admission and highlights the benefit of longitudinally monitoring SG subtypes in trauma patients.

KEYWORDS—Longitudinal, prognosis, subtype, transcriptome, trauma

INTRODUCTION

Trauma contributes to 10% of deaths and 16% of disabilities worldwide (1). Furthermore, the diversity in clinical trajectories (rapid resolution vs. multiorgan dysfunction) points to heterogeneity in the immune response among critically ill trauma patients (2). A large-scale gene array study first demonstrated a cytokine storm after systematic injury in the whole leukocyte transcriptome and that complicated clinical courses are associated with prolonged immune dysregulation (3). We provided the first single-cell RNA-sequencing data of circulating mononuclear cells derived from 10 trauma patients sampled at less than 4, 24, and 72 hours after injury and paired control subjects (4). In this study, we showed that monocytes underwent dramatic changes after injury, and the transcriptomic patterns in human blood $CD14^+$ monocytes could be generalized into six signatures that identified two patient subtypes (SG1 vs. SG2) using whole-blood leukocyte transcriptome data from 167 patients at 12 hours after injury. The major transcriptomic differences between SG1 and SG2 are in signature genes for inflammation, major histocompatibility complex II expression, and interferon signaling. Compared with SG2 patients, SG1 patients show an upregulation of proinflammatory genes and a simultaneous suppression of genes representing major histocompatibility complex II expression and interferon signaling. Clinically, patients classified as SG1 had significantly worse outcomes than patients classified as SG2. To better understand the potential prognostic value of SG subtype designation over time, we sought to characterize the longitudinal changes in SG subtypes in association with a range of clinical outcomes.

METHODS

This study is a secondary analysis of a published data set. The transcriptomic data set generated from whole-blood leukocytes obtained from severely injured humans was downloaded from Gene Expression Omnibus (GSE36809). The cohort included 167 patients (18–55 years old) who had hypotension or acidosis and needed resuscitation from seven US hospitals. The corresponding clinical information was obtained from the lead author of the original article (3). Samples annotated as either "low RNA quality" or "incomplete time points" in metadata were excluded from the following analyses. The sampling time points were binned into the nearest time point, for a total of seven time bins: 12 hours, 1 day, 4 days, 7 days, 14 days, 21 days, or 28 days. If a patient had more than one time point falling in the same time bin, only the first time point in the bin was analyzed.

SG subtypes were computed for each data point (each patient at a specific time point) as we reported previously (4). Briefly, the classifier to assign SG subtypes was a random forest classifier based on the relative expression of six separate gene signatures (six intrinsic signature scores) within circulating leukocytes from a specific patient. These six signatures include three signatures (C1–C3) that are upregulated after trauma and three signatures (C4–C6) that are suppressed after trauma.

Kaplan-Meier (K-M) analysis was performed using the *survival* R package (v3.1.8) to evaluate 28-day recovery between (i) SG subtypes at a specific time point or (ii) longitudinal SG subgroups. The time-to-event data at a specific time point were defined as the time-to-recovery (TTR) from the original data minus the analyzed sampling time point. Recovery was defined by the original article (3). Briefly, recovery criteria from five organs were as follows: (1) cardiovascular: MAP >60 mm Hg and no inotropic/vasopressor support; (2) hematologic: platelet count >120,000/mL; (3) hepatic: serum bilirubin <3 mg/dL; (4) renal: no dialysis and creatinine <1.3 mg/dL; and (5) respiratory: no mechanical ventilation or ratio of arterial oxygen partial pressure (Pa_{O_2}) to fraction of inspired oxygen >300. Time to recovery was defined as the first day meeting all recovery criteria above without further organ system failure in any following days. For longitudinal SG subgroups, the later sampling time point (in the time bin of 1 day) was used as time zero. For nonsurvivors, "hospital length of stay" minus the analyzed sampling time point was used as the time-to-event data, and recovery status was annotated as

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"no." Patients who recovered before the analyzed sampling time point were excluded. To compare other variables between groups, continuous variables were depicted as median ± median absolute deviation and tested by Wilcoxon rank-sum test. Categorical variables were depicted as counts (percentages) and tested by Fisher exact test. A two-sided P value was computed. $P < 0.05$ was considered as significant.

This is a secondary analysis of a published data set. All data were deidentified, and the authors have no access to information that would link the information to the subjects. Therefore, the authors' analysis is not considered human subjects research and therefore does not require institutional review board approval.

RESULTS

SG subtype designation displayed dynamic changes after trauma within 12 hours to 7 days postinjury, with a general trend to transition from SG1 to SG2 over time (Fig. 1A). The 12 hours' time point had the highest percentage of SG1 patients. Approximately 50% of patients identified as SG1 at 12 hours converted to SG2 by 1 day, a trend that continued at later time points. However, there were also a small number of patients who switched from SG2 to SG1 between 12 hours and 1 day and over time. By 4 to 7 days postinjury, subgroup designation remained relatively stable in surviving patients.

To investigate the potential prognostic value of SG status at later time points, we performed K-M analyses at each time point separately. Because of limited numbers of SG1 patients after 7 days, we analyzed only the data points in the time bins ≤7 days. SG1 designation was consistently associated with a subsequent slower recovery (Fig. 1, B–D).

The finding that some patients converted from SG2 to SG1 at 1 day suggested that assessing SG status at both 12 hours and 1 day could improve prognostic accuracy. Next, we performed K-M analysis in subgroups of patients based on the SG status at either 12 hours or 1 day (Fig. 2). For the patients who were SG1 at one time point, the SG status at another time point showed a trend without achieving statistical significance. However, for patients who were classified as SG2 at 1 day and SG1 at 12 hours, the recovery was dramatically slower than those classified as SG2 at both time points ($P = 0.0024$). A similar pattern was seen in the patients who were SG2 at 12 hours but then became SG1 at 1 day ($P = 0.073$).

In addition to TTR, a measure of global recovery from organ dysfunction, we also examined other outcome variables (Supplemental Tables 1 and 2, http://links.lww.com/SHK/B467, http://links.lww.com/SHK/B468) and potential associated factors (Supplemental Tables 3 and 4, http://links.lww.com/SHK/B469, http://links.lww.com/SHK/B470) in subgroup analysis. Generally, in the four subgroups that included patients defined as SG1–>SG1, SG2–>SG2, SG1–>SG2 or SG2–>SG1, being SG1 at either 12 hours

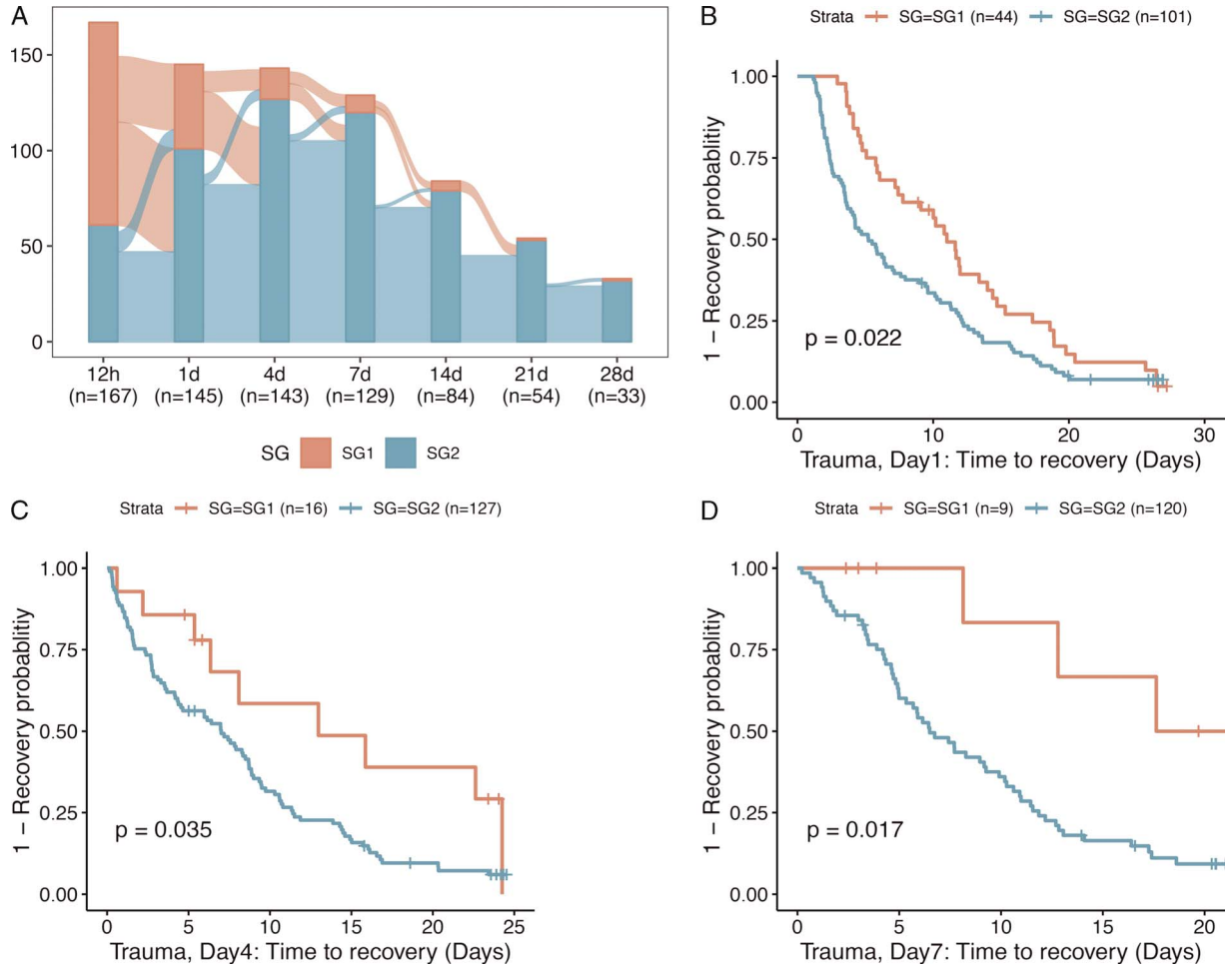

FIG. 1. **Prognostic value of SG subtypes at 1, 4, and 7 days postinjury.** A, Overview of SG subtypes over time after injury. Sampling time points were binned in the nearest time bin. For the patients with more than one time point in a time bin, the first time point was kept. B–D, K-M analysis at each later time point. Log-ranked P value was provided. B, Patients were grouped by the SG status at 1 day. C, Patients were grouped by the SG status at 4 days. D, Patients were grouped by the SG status at 7 days. Time zero for K-M analysis was set as the analyzed time point.

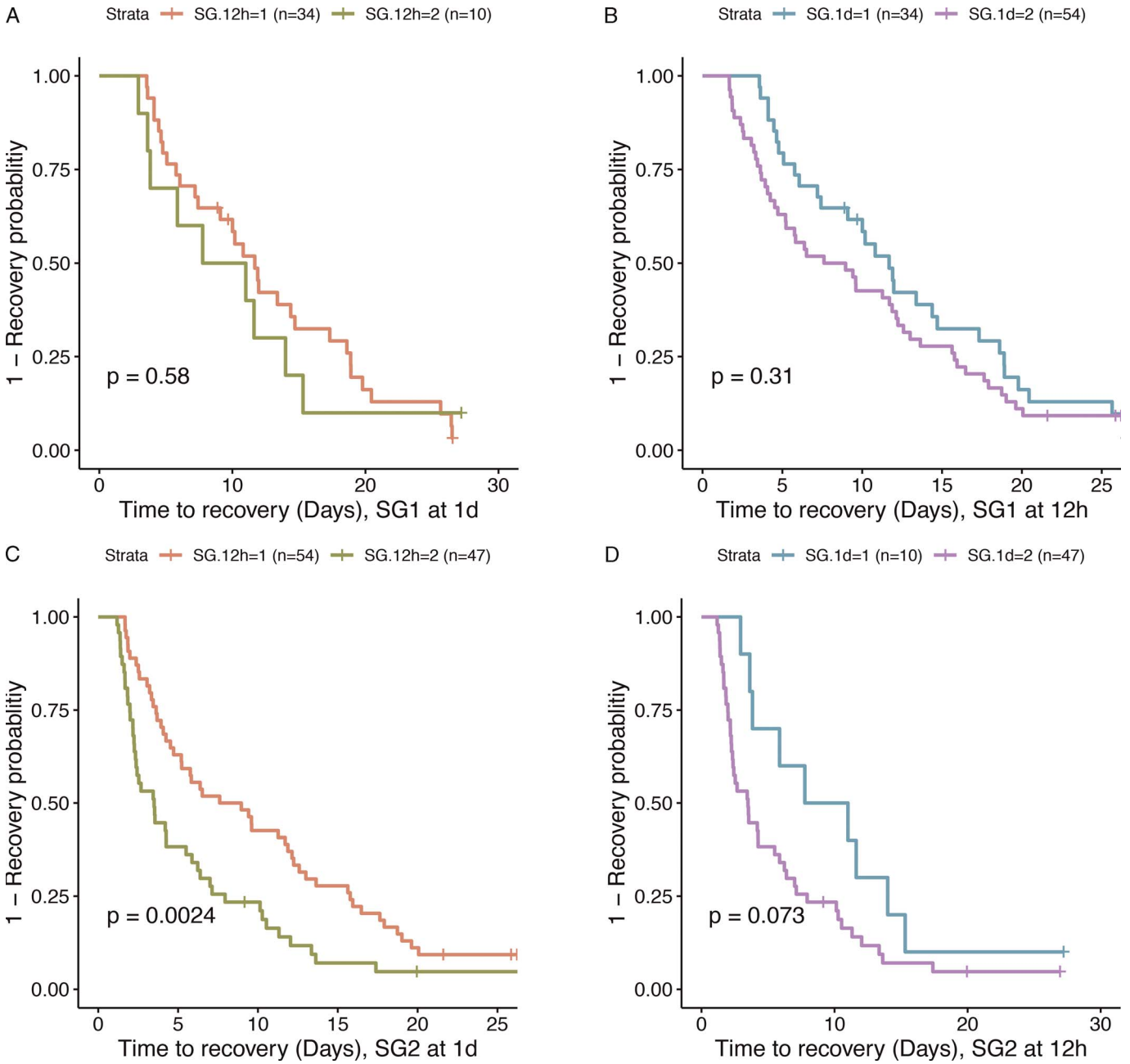

Fig. 2. **Kaplan-Meier analysis in subgroup analysis.** Patients were divided into subgroups into their SG status at either 12 hours or 1 day. Log-ranked *P* value was provided. A, Of all SG1 patients at 1 day, the prognostic value of SG status at 12 hours was evaluated. Time zero for K-M analysis was set as the time point falling in the bin of 1 day. B, Of all SG1 patients at 12 hours, the prognostic value of SG status at 1 day was evaluated. C, Of all SG2 patients at 1 day, the prognostic value of SG status at 12 hours was evaluated. D, Of all SG2 patients at 12 hours, the prognostic value of SG status at 1 day was evaluated.

or 1 day was generally associated with worse outcomes across the variables. Especially, among the patients who were SG2 at either 12 hours or 1 day, the patients who were SG1 at the other time point exhibited significantly longer ICU and hospital stays, as well as more days on the ventilator. These findings show that an SG1 designation at either time point has additional prognostic value beyond using either time point alone.

Interestingly, among SG2 patients at 12 hours, patients who converted to SG1 at 1 day showed higher heart rate, higher respiratory rate, lower PaO_2, and lower systolic blood pressure after arrival, suggestive of cardiovascular dysfunction (Supplemental Table 3, http://links.lww.com/SHK/B469). This association was not found in other subgroups. These patients also showed higher cardiac Marshall scores and delayed cardiac recovery (Supplemental Table 1, http://links.lww.com/SHK/B467). These results suggest that early cardiovascular dysfunction may be a factor for the conversion from SG2 to SG1 between 12 and 24 hours postinjury. The transcriptomic profile shifting away from a favorable state to an unfavorable state early after arrival points to the importance of monitoring transcriptomic subtypes over time should this strategy be adopted for clinical decision support. In addition, our findings support that the prevention or reversal of hemodynamic instability (higher heart rate, higher respiratory rate, lower PaO_2, and lower systolic blood pressure) after admission is of paramount importance to minimize immune dysfunction (5,6). Admittedly, there are only 10 patients of SG2–>SG1, and some patients also have

missing values. Thus, this hypothesis needs further validation, and we cannot exclude the possibility that emergence of immune dysfunction played a role in the persistent hemodynamic instability.

Previously, we showed that SG1 designation at 12 hours only correlated significantly with a limited number of known prognostic factors, including higher Injury Severity Score and higher lactate levels in the initial 6 hours. After adjusting for potential covariants using Cox regression model, we demonstrated that SG status at 12 hours has independent prognostic value beyond known prognostic factors (4). In contrast, SG1 status at 1 day was correlated significantly with more known prognostic factors, including higher Acute Physiology and Chronic Health Evaluation II score, higher crystalloids, blood transfusion during the first 12 hours, and higher lactate and lower base deficit at later time points within 24 hours postinjury (Supplemental Table 5, http://links.lww.com/SHK/B471). These results further highlight that the assessment of SG status at 12 hours is unique as a prognostic indicator.

To visualize the prognostic value of SG status at both 12 hours and 1 day, we divided all the patients with data available at both 12 hours and 1 day into four groups, labeled as SG status at 12 hours –> at 1 day, including two groups of SG nonconverters (SG1–>SG1 or SG2–>SG2) and two groups of converters (SG1–>SG2 or SG2–>SG1) (Supplemental Figure 1, http://links.lww.com/SHK/B472). For categorical outcomes (nosocomial infections and noninfectious complications), SG1 nonconverters had the highest incidence, and SG2 nonconverters had the lowest incidence. The two groups of converters showed an intermediate incidence of these outcomes. Similar patterns were observed in global recovery. SG1 nonconverters underwent the slowest recovery, followed by the two groups of converters, and then SG2 nonconverters with the fastest recovery. Notably, all SG1 nonconverters exhibited a TTR of 5 days or greater. Thus, monitoring SG subtype at two time points over the first day may lead to a high capture of patients likely to experience slow recovery and organ dysfunction.

DISCUSSION

This study was undertaken to extend our previous findings using a patient classifier developed from transcriptomic patterns identified in circulating $CD14^+$ monocytes from injured humans (4). Using transcriptomic data from bulk leukocytes, we were able to use the classifier to designate patients as SG1 (worse prognosis) or SG2 (better prognosis) at multiple time points after injury. We confirmed that patient designation as SG1 as early as 12 hours after injury correlated strongly with slow recovery. We went on to show that a subset of patients with early hemodynamic instability converted from SG2 to SG1 by 1 day, showing that unstable patients can evolve to a pathogenic gene signature after admission. Finally and most importantly, monitoring SG subtype at two time points over the first day can provide additional prognostic value beyond single time point measurements and lead to a very high capture of patients likely to experience slow recovery and organ dysfunction.

Interestingly, the patients who converted from SG2 to SG1 were associated with worse cardiovascular parameters (higher heart rate and lower blood pressure) early postinjury. Thus, the switch from SG2 to SG1 within day 1 may result from cardiovascular dysfunction, traumatic shock, or ongoing bleeding. The idea that the transcriptomic profile can shift away from a favorable state to an unfavorable state early after arrival points to the importance of monitoring transcriptomic subtypes over time should this strategy be adopted for clinical decision support. In addition, our findings confirm that the prevention or reversal of hemodynamic instability after admission is of paramount importance to minimize immune dysfunction (5,6).

There are two main limitations of this study. First is the absence of another large-scale data set for secondary validation. This trauma data set is the only longitudinal trauma data set incorporating whole-blood leukocyte transcriptomic data with enough subjects and adequate clinical information, including time-to-event data, needed for this type of analysis. The second limitation is the limited number of patients (n = 10) in the subgroup SG2–>SG1. Another interpretation of this finding is that frequency of this clinical trajectory is relatively small. A number of additional steps would be required to advance these findings into the clinical setting. These include the identification of the minimum number of genes needed to classify patients as SG1 or SG2 and a prospective study to validate the prognostic accuracy of SG designation.

We have demonstrated previously that SG subtypes also associate with a dysfunctional immune response across several etiologies of critical illness, including sepsis and burns (4). Thus, longitudinally monitoring transcriptomic subtypes in other etiologies of critical illnesses (7,8) may also be helpful and warrant further exploration.

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