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Plasma proteomics profile-based comparison of torso versus brain injury: A prospective cohort study

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BACKGROUND: Trauma-related deaths and posttraumatic sequelae are a global health concern, necessitating a deeper understanding of the pathophysiology to advance trauma therapy. Proteomics offers insights into identifying and analyzing plasma proteins associated with trauma and inflammatory conditions; however, current proteomic methods have limitations in accurately measuring low-abundance plasma proteins. This study compared plasma proteomics profiles of patients from different acute trauma subgroups to identify new therapeutic targets and devise better strategies for personalized medicine.

METHODS: This prospective observational single-center cohort study was conducted between August 2020 and September 2021 in the intensive care unit of Osaka University Hospital in Japan. Enrolling 59 consecutive patients with blunt trauma, we meticulously analyzed plasma proteomics profiles in participants with torso or head trauma, comparing them with those of controls (mild trauma). Using the Olink Explore 3072 instrument (Olink Proteomics AB, Uppsala, Sweden), we identified five endotypes (α–ε) via unsupervised hierarchical clustering.

RESULTS: The median time from injury to blood collection was 47 minutes [interquartile range, 36–64 minutes]. The torso trauma subgroup exhibited 26 unique proteins with significantly altered expression, while the head trauma subgroup showed 68 unique proteins with no overlap between the two. The identified endotypes included α (torso trauma, n = 8), β (young patients with brain injury, n = 5), γ (severe brain injury postsurgery, n = 8), δ (torso or brain trauma with mild hyperfibrinolysis, n = 18), and ε (minor trauma, n = 20). Patients with torso trauma showed changes in blood pressure, smooth muscle adaptation, hypermetabolism, and hypoxemia. Patients with traumatic brain injury had dysregulated blood coagulation and altered nerves regeneration and differentiation.

CONCLUSION: This study identified unique plasma protein expression patterns in patients with torso trauma and traumatic brain injury, helping categorize five distinct endotypes. Our findings may offer new insights for clinicians, highlighting potential strategies for personalized medicine and improved trauma-related care. (*J Trauma Acute Care Surg.* 2024;97: 557–565. Copyright © 2024 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Association for the Surgery of Trauma.)

LEVEL OF EVIDENCE: Prognostic and Epidemiological; Level III.

KEY WORDS: Coagulopathy; endotypes; plasma proteomics; tissue hypoperfusion; trauma.

Recently, approximately 4.5 million trauma-related deaths and tens of millions of posttraumatic sequelae have been reported annually worldwide;[1] reducing the number of these trauma-related deaths and sequelae is a universal challenge. Understanding the complex pathophysiology of trauma is essential to developing new treatment modalities, leading to advancements in trauma therapy. To elucidate the molecular mechanisms underlying various disease states, researchers have used proteomics approaches to comprehensively identify proteins associated with these conditions and to analyze their functions and interactions to redefine these diseases.[2–4]

Along with other recent technological advancements, proteomics has facilitated the semiquantitative measurement of several plasma proteins.[5] Mass spectrometry has been used in many fields, such as peptide-based nonspecific proteomics.[6–8] However, it is limited by the difficulty in examining low-abundance and low-molecular-weight fractions of the human plasma proteome, such as inflammatory cytokines.[9] A new specific proteomics approach combining immunoassay and protein-sequencing technologies has overcome this limitation and allowed the semiquantitative measurement of several plasma proteins.[10] The plasma proteomes of trauma and acute inflammatory infection cases were previously evaluated to elucidate novel molecular pathogenetic mechanisms.[11,12] However, only a limited number of studies have focused on the semiquantitative measurement or molecular pathogenetic analysis of several plasma proteins in trauma patients with high accuracy. A comprehensive search

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for plasma proteins whose expression is regulated by trauma can provide mechanistic insights into the complex pathology of this condition.[13]

Hence, this study aimed to elucidate the molecular pathogenesis of acute trauma by screening and comparing expression levels of various relevant plasma proteins in the acute phase of torso trauma and traumatic brain injury (TBI) to identify new therapeutic targets and to devise better strategies for personalized medicine.

PATIENTS AND METHODS

Study Design and Settings

This prospective cohort study was conducted at a single center, which encounters approximately 250 severe trauma cases per year (see Supplemental Digital Content, Supplementary Data 1, http://links.lww.com/TA/D725, for further information on the research facility). This study followed the STrengthening the Reporting of OBservational studies in Epidemiology guidelines, http://links.lww.com/TA/D726.[14] It was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the center (institutional review board approval number, 885; approval date, June 29, 2020; novel molecular pathogenesis of systemic inflammatory diseases based on comprehensive biomolecular information). All study participants provided written informed consent, and no stipend was provided to them.

Study Population

Adult patients (18 years or older) admitted to our center by emergency transport between August 2020 and September 2021 were included. The exclusion criteria were as follows: (1) cases transferred from other hospitals, (2) cases of cardiopulmonary arrest on arrival, (3) cases with Injury Severity Scores (ISSs)[15] of 75 points, (4) cases of early death (within 24 hours of injury), (5) cases of confirmed refusal to continue life-sustaining treatment, (6) pregnant and parturient women, and (7) cases of trauma with an Abbreviated Injury Scale (AIS)[16] score of ≥4 points to the head and torso. For a comparative analysis of patients with minor trauma, torso trauma, and isolated TBI, patients were selected according to the following criteria: patients in the minor-injury group did not have trauma with an AIS score of ≥3 points to the head or torso; those in the torso trauma group had trauma with an AIS score of ≥3 points to the chest or abdomen only and had no trauma with an AIS score of ≥3 points to the head; and those in the TBI group had head trauma with an AIS score of ≥3 points as the primary trauma. For proteomic analysis, we selected patients consecutively admitted to the hospital such that the ratio of the number of cases in the three groups was approximately 1:1:2. Plasma proteomics profiling was conducted using patient plasma in the early postinjury period using Olink Explore 3072 (Olink Proteomics AB, Uppsala, Sweden; Supplemental Digital Content, Supplementary Table 1, http://links.lww.com/TA/D726).

Plasma Sampling and Proteomic Profiling

Blood samples were collected within 1 hour of arrival at the hospital for general laboratory testing and proteomic analyses. The samples for proteomic analysis were separated into plasma and stored at −30°C until analysis. Proteomic analysis for biomarker profiling was performed using the Olink Explore 3072 instrument. The proximity extension assay technology (PEA) used in Olink Explore 3072 can detect approximately 3,000 proteins. The PEA combines DNA-tagged antibodies and polymerase chain reaction to enhance the sensitivity and specificity of protein detection, enabling the identification of low-abundance proteins[5] (for a more detailed explanation on proteomics, see the eMethods in Supplemental Digital Content, Supplementary Data 1, http://links.lww.com/TA/D726).

The protein levels measured are expressed as normalized protein expression (NPX) value in the log2 scale.[5] The NPX value is positively correlated with protein concentration—a one-fold numeral increase in the NPX value indicates a twofold increase in the protein concentration in the sample.

Statistical Analyses

Data collection is described in Supplemental Digital Content (Supplementary Data 1, http://links.lww.com/TA/D726). Patient characteristics are described as means (SDs) or medians (interquartile ranges [IQRs]), as appropriate. When comparing baseline patient characteristics, Pearson's χ^2 test was used for categorical data, and the Kruskal-Wallis test was used for continuous data. Welch's *t* test was performed to assess significant changes in protein expression between two subgroups. To assess the impact of torso trauma included in the TBI group, a subgroup analysis targeting this trauma was conducted. The Benjamini-Hochberg method[17] was used to adjust for multiple testing to provide false discovery rate (FDR)–adjusted *p* values, with statistical significance set at <0.1.

A volcano plot was created to visualize proteins differentially expressed between the minor trauma and other trauma subgroups; the *x* axis of the graph represents the difference in NPX values, and the *y* axis represents −log10 (*p* value). Proteins with FDR-adjusted *p* values of <0.05 were plotted in red, while proteins with *p* values of ≥0.05 and <0.1 were plotted in purple. Unsupervised hierarchical clustering was used to check whether endotypes could be derived using only protein information that changed significantly with torso trauma and TBI. To confirm the validity of the clustering, t-distributed stochastic neighbor embedding (t-SNE)[18] was performed as a sensitivity analysis. Unless otherwise noted, the threshold for statistical significance using a two-tailed test was <0.05. This was a preliminary study, and we did not use a formal power analysis for sample size determination; rather, the sample size was guided by pragmatic constraints, including available data, time, budget, and ethical considerations. These findings should be regarded as preliminary, providing direction for hypothesis generation and informing future confirmatory studies. All statistical analyses were performed using R version 4.0.2 (R Software for Statistical Computing, Vienna, Austria).

RESULTS

Study Population

During the study period, 208 patients with blunt trauma were transported and consecutively admitted to our center. After excluding 149 patients who did not meet the inclusion criteria, 59 were eventually included. Of these, 15 had minor trauma, 15 had torso trauma, and 29 had TBI. Supplemental Digital Content (Supplementary Table 2, http://links.lww.com/TA/D726) presents the baseline patient characteristics of these three groups.

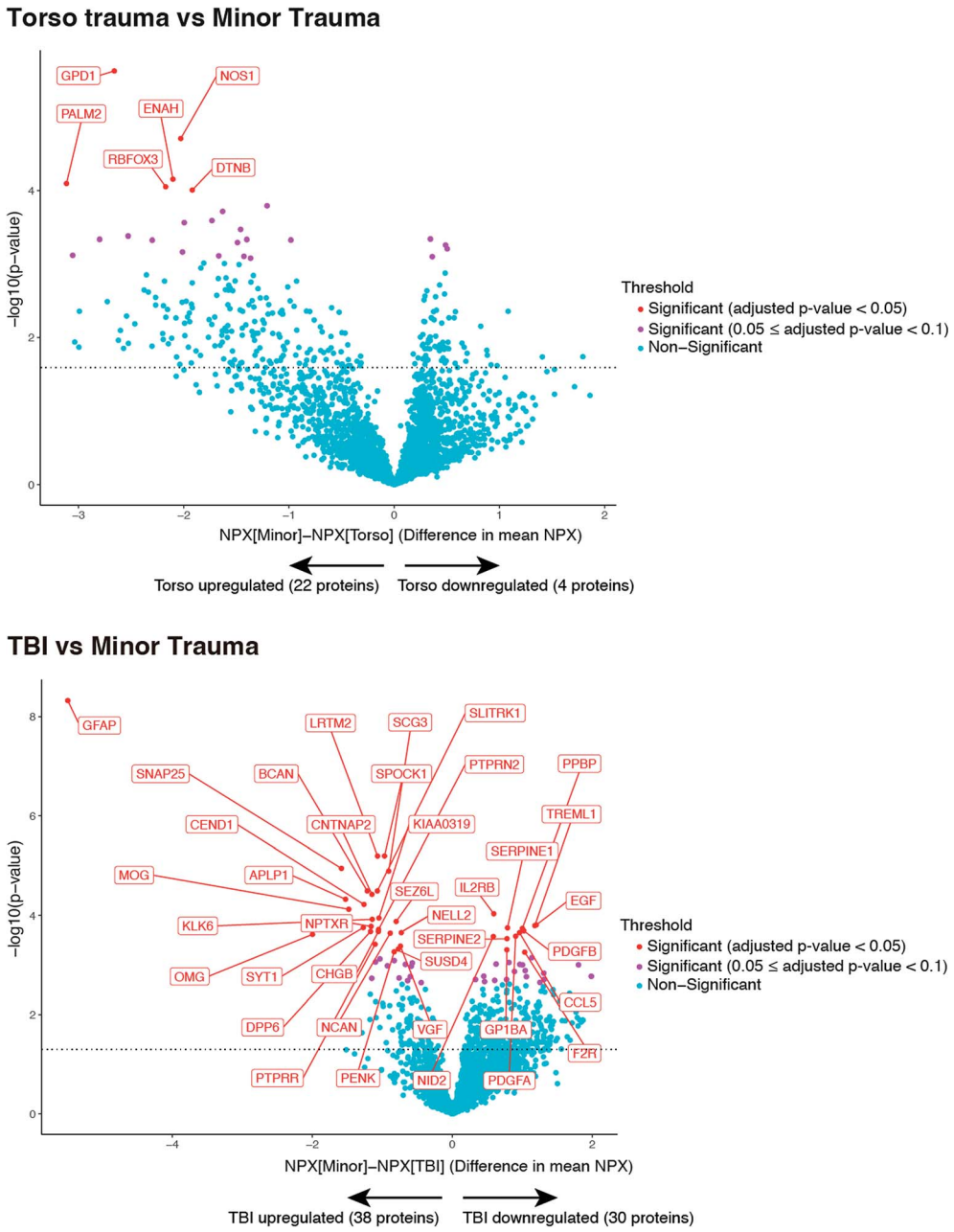

Figure 1. Volcano plot: torso trauma and TBI showing differences in plasma protein expression. The vertical axis shows the negative logarithm of the real number of *p* values, and the horizontal axis shows the difference in NPX values. Proteins with an FDR-adjusted *p* value of <0.05 are plotted in red, and those with an FDR-adjusted *p* value of ≥0.05 and <0.1 are plotted in purple. Minor trauma is used as a control; the upper left plot shows proteins that are upregulated in each injury, and the upper right plot shows downregulated proteins.

There were no missing values for any variables. Overall, the median age was 51 years [IQR, 45–70 years], 43 participants were men (72.9%), the median ISS was 22 points [IQR, 10–26 points], and the in-hospital mortality rate was 8.5%. Age and sex were not significantly different among the three groups. There were significant differences in systolic blood pressure and Glasgow Coma Scale scores[19,20] ($p = 0.003$ and $p < 0.001$, respectively). Other vital signs were not significantly different among the three groups.

No head or torso trauma with an AIS score of ≥3 points was noted in the minor trauma group. There were no TBIs with an AIS score of ≥3 points in the torso trauma group. In addition, six (21%) and three patients (10%) with chest and abdominal trauma, respectively, with an AIS score of ≥3 points were noted in the TBI group. Among the three groups, hemoglobin concentration, platelet count, serum lactate level, and in-hospital mortality were not significantly different, although fibrin/fibrinogen degradation product and D-dimer (both $p < 0.001$) levels and ventilator management and blood transfusion within 24 hours of injury ($p < 0.001$ and $p = 0.002$, respectively) were significantly different.

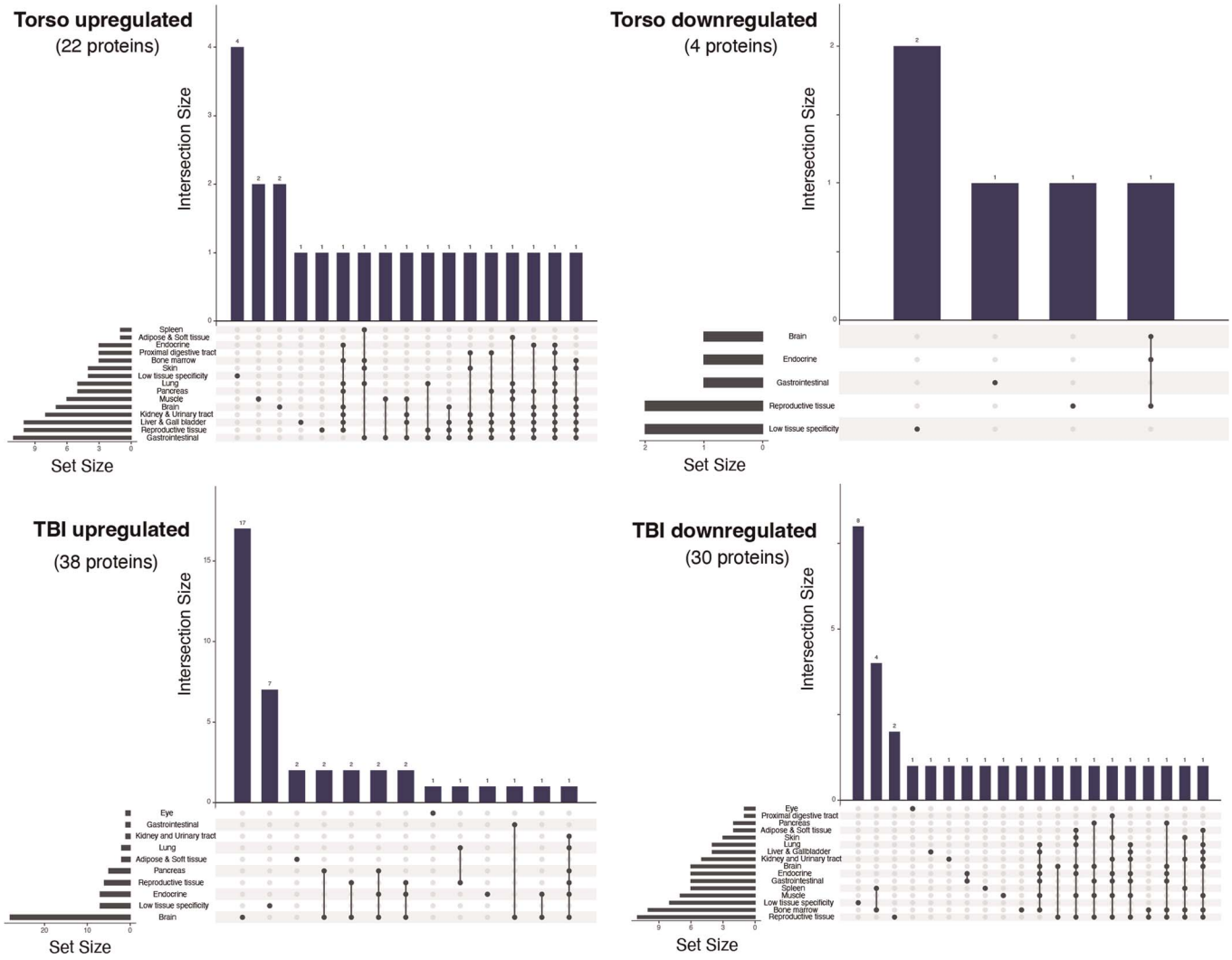

Figure 2. Tissue enrichment: significantly altered protein levels in torso trauma are expressed primarily in the gastrointestinal tract and reproductive organs; upregulated proteins in TBI are primarily expressed in the brain, and downregulated proteins are expressed in the reproductive organs and bone marrow. Protein characterization data are obtained from the Human Protein Atlas.

Protein Profiles

The median time from injury to blood collection was 47 minutes with [IQR, 36–64 minutes]. We evaluated changes in plasma protein expression in the torso trauma and TBI groups while using minor trauma cases as controls. With an FDR significance threshold of <0.1, we found significant changes in the expression of 26 proteins in the torso trauma (Supplemental Digital Content, Supplementary Table 3.1, http://links.lww.com/TA/D726; 22 upregulated and 4 downregulated) and 68 in the TBI (Supplemental Digital Content, Supplementary Table 3.2, http://links.lww.com/TA/D726; 38 upregulated and 30 downregulated) groups compared with those in minor trauma. Volcano plots are presented in Figure 1; all proteins differentially expressed in the torso trauma and TBI groups were unique to each group. With an FDR significance threshold of <0.05, GPD1, NOS1, ENAH, PALM2, RBFOX3, and DTNB were upregulated in the torso trauma group, while 38 proteins (GFAP, SPOCK1, LRTM2, SNAP25, SCG3, BCAN, SLITRK1, CNTNAP2, APLP1, CEND1, MOG, IL2RB, KIAA0319, KLK6, SEZ6L, EGF, TREML1, NPTXR, SYT1, SERPINE1, PPBP, PTPRN2, PDGFB, DPP6, CHGB, CCL5, NELL2, NCAN, OMG, PDGFA, NID2, SERPINE2, PTPRR, VGF, SUSD4, GP1BA, PENK, and F2R) showed significant changes in the TBI group (26 upregulated and 12 downregulated). To evaluate the impact of torso trauma in the TBI group, a subgroup analysis was performed on these patients (6 of 29 patients with TBI; Supplemental Digital Content, Supplementary Table 3.3, http://links.lww.com/TA/D726). Using an FDR significance threshold of <0.1, significant changes were observed in 36 proteins, of which 3 were proteins that had shown significant changes in the analysis of torso injuries.

Tissue Enrichment

With an FDR significance threshold of <0.1, the 22 upregulated proteins in torso trauma were expressed in the gastrointestinal tract, genital tract, and liver or gallbladder in descending order of magnitude; the four downregulated proteins were either expressed in the genital tract or were less tissue specific.[21] In the TBI group, most upregulated proteins (38 proteins) were

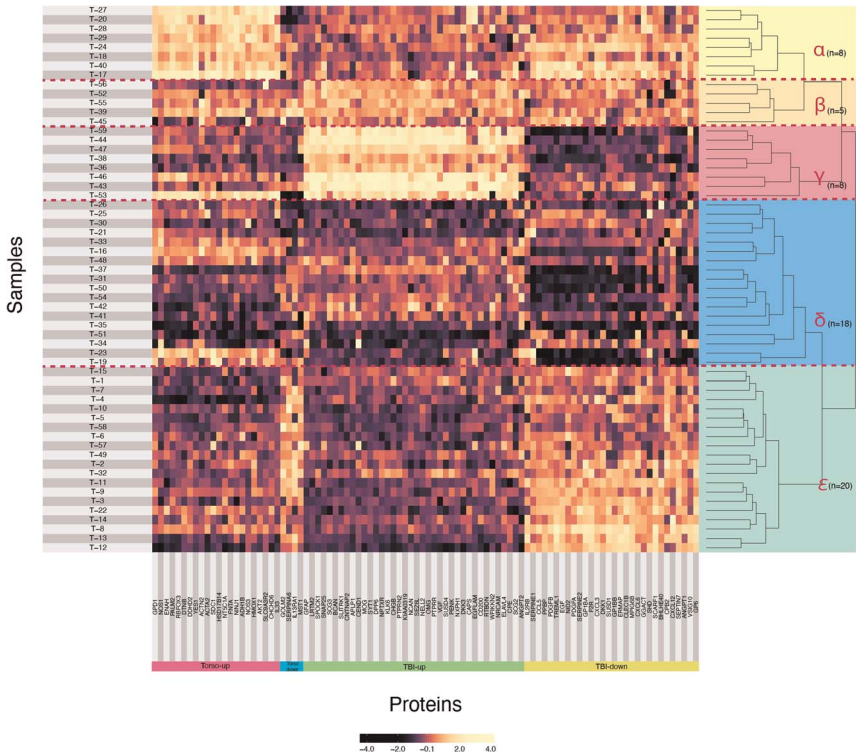

Figure 3. Heatmap generated by unsupervised hierarchal clustering: unsupervised hierarchical clustering is conducted using proteins with significantly altered expression in torso trauma and TBI compared with minor trauma (torso trauma, 26 proteins; TBI, 68 proteins), and this analysis generates five endotypes (α–ε). Lighter colors indicate more strongly expressed proteins.

expressed exclusively in the brain, and the downregulated proteins (30 proteins) were mainly expressed in the reproductive and immune systems (bone marrow, spleen) and muscle, among other tissues (Fig. 2).

Hierarchal Clustering

To verify whether case-specific protein expression levels were unaffected and to elucidate disease subtypes, we performed an unsupervised hierarchical cluster analysis of proteins with significantly altered expression in the torso trauma and TBI groups. This approach revealed five endotypes, α–ε (Fig. 3): α (n = 8, patients with torso trauma) comprised a distinct group of proteins upregulated in the trunk; β (n = 5, young patients with brain injury) comprised moderately elevated expression of proteins upregulated in TBI cases; γ (n = 8, patients with severe brain injury) showed a marked increase in the expression of proteins upregulated in TBI and a clear decrease in the expression of proteins downregulated in TBI; δ (n = 18, patients with

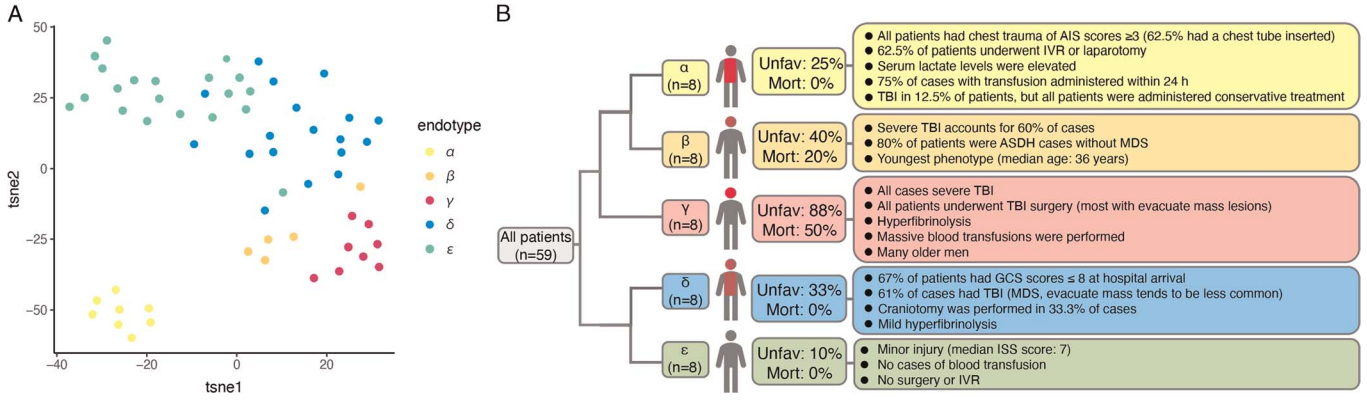

Figure 4. (*A*) t-Distributed stochastic neighbor embedding plot: endotypes derived using hierarchical clusters are displayed using the t-SNE plot. (*B*) Description of the five endotypes: clustering based on significantly altered protein expression divides the patients into five clusters according to their protein expression, and these clusters generally reflect the site of injury and its severity. The proportion of patients in each cluster with a poor prognosis and cluster mortality are also shown. Pictograms show the main injury locations, with darker red indicating more severe injuries. Unfav, unfavorable outcome rate (defined as a Glasgow Coma Scale score of ≤3 points). ASDH, acute subdural hematoma; GCS, Glasgow Coma Scale; IVR, interventional radiology; MDS, midline shift; Mort, mortality.

torso or brain trauma) showed decreased expression of TBI downregulated and upregulated proteins similar to that in the control group; and ε (n = 20, patients with minor trauma) showed minor variation in the expression of proteins significantly altered in the TBI and torso trauma groups.

t-SNE Plot

To confirm the validity of hierarchical clustering, t-SNE analysis was conducted as a sensitivity analysis to visualize cluster separation (Fig. 4*A*). The t-SNE results confirmed that groupings were consistent with hierarchical clustering findings.

Description of the Five Endotypes

Supplemental Digital Content (Supplementary Table 4, http://links.lww.com/TA/D726) and Figure 4*B* show the characteristics of each cluster. The α endotype (n = 8) was characterized by the presence of torso trauma. All patients with chest trauma had an AIS score of ≥3 points (62.5% had a chest drain inserted), and five (62.5%) patients underwent interventional radiology or open hemostasis. One patient had an acute epidural hematoma that was treated conservatively. The β endotype (n = 5) was represented by the smallest cluster and comprised patients with TBI (median age, 36 years [IQR, 32–47 years]): three (60%) with severe TBI and four (80%) with acute subdural hematoma without a midline shift. Three patients (60%) underwent surgery for TBI. The unfavorable outcome and in-hospital mortality rates were 40% and 20%, respectively. The γ endotype (n = 8) mostly comprised older men and was characterized by severe TBI and surgery for TBI. Blood tests showed hyperfibrinolysis; all patients received a significant amount of blood transfusions. The worst prognosis was in the poorest cluster (γ endotype), with 88% poor neurological prognosis and 50% in-hospital mortality rates. In the δ endotype (n = 18), 12 patients (67%) had a Glasgow Coma Scale score of ≤8 points, 11 (61%) had TBI with an AIS score of ≥3 points, 6 (33%) underwent craniotomy, and 7 (39%) had torso trauma with an AIS score of ≥3 points. Hyperfibrinolysis was mild. The ε endotype (n = 20) was characterized by a median ISS of 7 points for all minor trauma cases, with no transfusion administration, interventional radiology, or surgery.

Enrichment Analysis

Gene Ontology enrichment analysis of proteins upregulated in torso trauma revealed pathways involved in smooth muscle adaptation, blood pressure regulation, hypermetabolism, and hypoxemia. The number of downregulated proteins in torso trauma was small; therefore, an enrichment analysis could not be performed. Proteins significantly upregulated in TBI were associated with nerve regeneration and differentiation. Proteins downregulated in TBI were associated with pathways that regulate blood coagulation and platelet activation, wound healing, and fluid regulation (Supplemental Digital Content, Supplementary Fig. 1, http://links.lww.com/TA/D726). Details of proteins involved in each pathway are summarized in Supplemental Digital Content (Supplementary Table 5, http://links.lww.com/TA/D726).

DISCUSSION

We performed a large-scale proteomic analysis to screen for protein classes implicated in trauma, allowing the parallel identification of changes in expression patterns of protein pathways in patients with torso trauma and those with TBI. Proteins with significantly altered expression differed widely between the torso trauma and TBI patient subgroups, indicating different pathophysiologies in these subgroups. We also derived five endotypes based on the significantly altered protein expression, which were clinically different and definable categories.

This study is one of the largest studies in the field of trauma to use highly accurate, high-throughput proteomics profiling. To date, proteomics has had technical limitations, such as the lack of sensitivity and specificity of analytical methods, limited multiplexing possibilities, and low sample throughput (processing power and data transfer volume). The PEA method[5] used in Olink Explore 3072 allows high-accuracy and enhanced detection of low-abundance proteins using DNA-tagged antibodies and polymerase chain reaction amplification.[22] The high-throughput proteome profiling described in this study enabled the capture of proteins altered during the acute phase of trauma with high accuracy.

The findings of this study provided three clinical implications. First, the patients in the torso trauma and TBI subgroups had differentially altered protein expression levels in the body. Torso trauma was characterized by pathways related to smooth muscle adaptation, blood pressure regulation, hypermetabolism, and hypoxemia. Conversely, TBI was characterized by pathways related to the blood coagulation system, platelet function, and nerve regeneration and differentiation. The finding that such pathways are active during the acute phase of trauma is interesting. The fundamental strategy in current trauma treatment focuses on optimizing organ perfusion early through surgical hemostasis and transfusions for preventing secondary injuries. However, while supporting these conventional treatment strategies,[23–26] our findings indicate that, particularly in TBI, pathways involved in neural regeneration and differentiation are activated during the acute phase. This could support the development of new treatment strategies and novel therapeutics aimed at promoting the neural regeneration and differentiation in the acute phase in response to neural tissue damage. However, the absence of overlapping differentially expressed proteins in torso trauma and TBI was contrary to our expectations. The possible interaction between different specific injury combinations in trauma-related deaths in multiple trauma cases was previously reported.[27] Our prior expectations were that torso trauma and TBI would lead to the identification of proteins with altered expression in both trauma subgroups. Furthermore, it may seem contradictory that no overlapping proteins were detected between the TBI group and those with torso trauma. Among the 29 cases in the TBI group, 6 of torso trauma with an AIS score of 3 were analyzed as a subgroup, and the results are presented in Supplemental Digital Content (Supplementary Table 3.3, http://links.lww.com/TA/D726) (TBI with torso trauma vs. minor trauma). Three proteins identified in the cases of torso trauma showed significant changes, and other proteins altered by torso trauma were ranked highly in terms of change. Therefore, it was speculated that, despite the presence of proteins altered by torso trauma in the TBI group, their overall proportion in all TBI cases was low, leading to their statistical minimization and resulting in no detection of overlapping proteins. By minimizing heterogeneity within each subgroup and conducting a broader screening of

protein types, future research may detect overlapping proteins between torso trauma and TBI.

Second, we identified specific proteins with altered expression reported to be associated with neurotrauma (GFAP,[28–30] SPOCK1,[31] SNAP25,[32] CEND1,[33,34] MOG,[35] KIAA0319,[36] SYT1,[37] SERPINE1,[38] PDGFB,[39] CCL5,[40,41] OMG,[42] SERPINE2,[43] VGF,[44,45] PENK,[46] and F2R[47]), along with many previously unreported proteins. This supports the validity of the methodology and measurement system used herein. Significantly altered protein expression levels found in this study are associated with neurological and other diseases, although none had been previously implicated in trauma. Therefore, these are considered potential candidates for novel trauma biomarkers. Furthermore, the ability to evaluate multiple proteins collectively within a single cohort and to assess protein pathways in the acute phase of trauma was a novel aspect of this study.

Third, we deduced five endotypes using significantly altered protein expression levels and provided different clinical explanations for each endotype. Each endotype was generally segregated by a combination of torso trauma and TBI severity. Differences between endotypes with the poorest prognosis, namely, the γ and δ endotypes, were significant: the γ endotype had elevated expression of proteins associated with and upregulated in TBI cases and showed decreased expression of proteins associated with and downregulated in patients with TBI; on the other hand, the δ endotype did not include any TBI-associated upregulated proteins but had decreased expression of proteins associated with and downregulated in TBI cases. Enrichment analysis showed that proteins upregulated in TBI were involved in nerve regeneration and repair, while proteins downregulated in TBI were involved in platelet function and the blood coagulation system. Thus, TBI with the δ endotype is considered a trauma that should be treated using treatment involving the correction of coagulation (blood transfusion strategy). In contrast, the γ endotype is characterized by elevated expression of proteins involved in nerve regeneration and repair, suggesting that a simple blood transfusion strategy would be insufficient; this appears consistent with actual neurological outcomes and patient prognoses in our cohort. Wu et al.[48] identified a subgroup of patients with TBI who benefited from prehospital thawed plasma administration; in our cohort, the δ endotype is considered similar to that in the previous study because blood transfusion is a common strategy. Considering the individual diversity of patients with trauma, the identification of endotypes from molecular pathology profiling may lead to personalized medicine and provide us with potential novel therapeutic targets and strategies.

Our study has some limitations. First, this study possessed selection bias, as only proteins registered in Olink Explore 3072 were analyzed. Moreover, all proteins identified by our proteomics profiling did not have a valid rationale in the field of trauma. In addition, the threshold for a significant change was set at an FDR of <0.1, which may be a type I error. However, this was intentionally done, as the primary purpose of this study was to describe alterations in protein levels after acute trauma that could provide insights for future studies from a broad perspective. We arbitrarily set the threshold for the FDR because we reasoned that a greater risk of type I error would be better than a greater risk of type II error, especially given the unknown statistical power required to detect differences in this new field of proteomics. To address the issue of overly sensitive parameters, we plan to conduct future research with increased sample size and reduced heterogeneity of the studied population, for example, by comparing subgroups based on more finely divided injured organs or using trauma phenotypes.[11]

Second, this study included patients with minor trauma as controls instead of healthy controls. In addition, the TBI group included some patients with torso trauma, potentially obscuring the distinct changes attributable to torso trauma versus TBI alone. This methodological approach was driven by the limited number of isolated TBI cases identified during the research period. For these patients, no surgical interventions were performed for chest or abdominal injuries, ensuring that the selection was limited to those with TBI as the primary concern. Although minor injuries did not result in significant clinical outcomes, they could have potentially obscured the true proteomic changes observed. Moreover, the absence of a minor TBI cohort as a comparator further limits the ability to detect proteomic changes, even in cases of significant head injury. Future studies using healthy controls or focusing exclusively on isolated TBI cases might reveal distinct protein profiles and enrichment analysis outcomes. Meanwhile, the lack of overlap in the proteins identified in the torso trauma and TBI subgroups using the minor trauma subgroup as the control may support the hypothesis that the molecular pathogeneses of torso trauma and TBI are quite different.

Finally, our study did not reveal a causal relationship between proteins that showed significantly altered expression and prognosis. Future basic molecular biology experiments in model organisms are needed to prove causality. Furthermore, our analysis was based on plasma protein data at a single time point after trauma and, therefore, may have excluded temporally modulated protein level changes that occurred before or after the sample collection point. In addition, the pathways showing significant changes were identified without considering the in vivo protein activity or performing protein-protein interaction evaluations.[49] Our interpretation of the data may change in the future, with improvements in techniques and our knowledge of proteome analyses, and as more data are accumulated over time.

CONCLUSION

In this prospective cohort study, we evaluated plasma proteomics profiles after torso trauma and TBI and identified 94 proteins with altered expression (26 in torso trauma and 68 in TBI) that were unique to patient subgroups, with no overlap. Unsupervised hierarchical clustering analysis using these proteins with significantly altered expression allowed the identification of five distinct endotypes. In torso trauma cases, the identified proteins were associated with smooth muscle adaptation, blood pressure regulation, hypermetabolism, and hypoxemia, while, in TBI cases, they were associated with blood coagulation abnormalities and nerve regeneration and differentiation. These molecular pathogenesis details revealed in each endotype via proteomic analysis may provide new ideas for trauma treatment strategies.

AUTHORSHIP

J.T. conceived and designed this study; contributed to the acquisition, analysis, and interpretation of the data; and was responsible for drafting,

editing, and submitting the manuscript. Y.T. made major contributions to data acquisition and interpretation of results. H.M. was involved in study conceptualization and significantly in data analysis, interpretation of the results, and manuscript preparation. T.M. contributed to the conceptualization of the study and made major contributions to data analysis, interpretation of results, and editing of the manuscript. S.S. advised on bioinformatics analysis methods and interpretation of the results. H.O. and J.O. provided significant input regarding data interpretation and critical appraisal of the manuscript. All authors contributed to data acquisition and reviewed, discussed, and approved the final version of the manuscript. All authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors had full access to all the data in the study and accept responsibility for publication of this manuscript.

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DISCLOSURE

Conflicts of Interests: Author Disclosure forms have been supplied and are provided as Supplemental Digital Content (http://links.lww.com/TA/D727).
Ethics Approval and Consent to Participate: The study was approved by the Ethics Committee of Osaka University (institutional review board approval number, 885; approval date, June 29, 2020; novel molecular pathogenesis of systemic inflammatory diseases based on comprehensive biomolecular information). All study participants provided written informed consent.
Availability of Data and Materials: The data and materials supporting the conclusions of this article are included in this published article. Primary Olink data and related data are available from the corresponding authors on request, although individual-level data can only be released under a suitable data-sharing agreement because of informed consent restrictions.

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