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SYMPATHOADRENAL ACTIVATION IS ASSOCIATED WITH ACUTE TRAUMATIC COAGULOPATHY AND ENDOTHELIOPATHY IN ISOLATED BRAIN INJURY

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ABSTRACT—**Background:** Acute coagulopathy after traumatic brain injury (TBI) involves a complex multifactorial hemostatic response that is poorly characterized. Objectives: To examine early posttraumatic alterations in coagulofibrinolytic, endothelial, and inflammatory blood biomarkers in relation to sympathetic nervous system (SNS) activation and 6-month patient outcomes, using multivariate partial least-squares (PLS) analysis. **Patients and Methods:** A multicenter observational study of 159 adult isolated TBI patients admitted to the emergency department at an urban level I trauma center, was performed. Plasma concentrations of 6 coagulofibrinolytic, 10 vascular endothelial, 19 inflammatory, and 2 catecholamine biomarkers were measured by immunoassay on admission and 24 h postinjury. Neurological outcome at 6 months was assessed using the Extended Glasgow Outcome Scale. PLS-discriminant analysis was used to identify salient biomarker contributions to unfavorable outcome, whereas PLS regression analysis was used to evaluate the covariance between SNS correlates (catecholamines) and biomarkers of coagulopathy, endotheliopathy, and inflammation. **Results:** Biomarker profiles in patients with an unfavorable outcome displayed procoagulation, hyperfibrinolysis, glycocalyx and endothelial damage, vasculature activation, and inflammation. A strong covariant relationship was evident between catecholamines and biomarkers of coagulopathy, endotheliopathy, and inflammation at both admission and 24 h postinjury. **Conclusions:** Biomarkers of coagulopathy and endotheliopathy are associated with poor outcome after TBI. Catecholamine levels were highly correlated with endotheliopathy and coagulopathy markers within the first 24 h after injury. Further research is warranted to characterize the pathogenic role of SNS-mediated hemostatic alterations in isolated TBI.

KEYWORDS—Catecholamines, D-dimer, hemostasis, norepinephrine, syndecan-1, thrombin-activatable fibrinolysis inhibitor, thrombomodulin, tissue factor pathway inhibitor, tissue plasminogen activator, vascular adhesion protein-1

INTRODUCTION

Traumatic brain injury (TBI) is a major cause of death and disability in both civilian and military populations (1). Although less common than mild head injuries (2), moderate-to-severe TBI is often life threatening (3), causing secondary injury complications that dramatically worsen patient outcome (4). Despite wartime advances in damage control resuscitation and surgery (5), trauma-associated coagulopathy in critically injured casualties remains an urgent concern both on and off the battlefield.

Acute traumatic coagulopathy is a global failure of the hemostatic system frequently reported in isolated TBI (6–8). The cited incidence of coagulopathy after TBI varies widely from 10% to 97% (9), but when present, coagulofibrinolytic derangements are strongly associated with increased mortality, transfusion requirements, organ failure, and hospital stay (8, 10). Both hypo- and hypercoagulable states have been

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Authors' contributions: SBR, SGR, AJB, KI, AB, and BBN were involved in the conception and design of the study. APD, BL, AM, and MYS acquired the data for the study. ALOM, APD, BL, MGH, HTP, and NC analyzed and interpreted the data. All authors were involved in drafting, revising, and providing intellectual contribution for the manuscript. All authors read and approved the final manuscript.

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described (11). Unfortunately, the pathophysiological mechanisms of acute trauma-induced coagulopathy are not well defined, owing to the complex underlying biology, limitations of current laboratory diagnostic tests (12, 13), and a lack of consensus on clinical terminology (14). Moreover, it is also unclear if coagulopathy in isolated TBI differs from multisystem trauma in either incidence (9, 15, 16) or pathogenesis (16, 17). To improve patient care and advance therapeutic options, a better understanding of acute coagulopathy and how it contributes to poor patient outcomes is needed (18).

Hemostasis involves the tightly regulated balance between coagulation and fibrinolysis that permits control of bleeding while preventing intravascular thrombosis (19). After injury, clotting is initiated when tissue damage exposes subendothelial tissue factor (TF) that activates coagulation factors to produce thrombin and fibrin (20). The brain is a rich source of procoagulant TF (21, 22), and although it is generally agreed upon that brain damage triggers elevated circulating TF levels and subsequent coagulopathy (22–24), there is no consensus of the sequelae that follow. Previous reports have suggested widespread TF release elicits disseminated intravascular coagulation (DIC), where the consumption of clotting factors leads to excessive bleeding (22, 23). However, others have refuted this, suggesting an immediate hypocoagulable response without the consumption of clotting factors, mediated by activation of protein C (24).

Recent studies in multisystem trauma suggest sympathetic nervous system (SNS) activation drives coagulopathy through endothelial damage/dysfunction, particularly by glycocalyx disruption (25–27). In addition, we recently demonstrated a link between SNS hyperactivity and inflammation in isolated TBI (28). Indeed, the reciprocal role of inflammation in coagulopathy is well characterized (29, 30); inflammatory cytokines promote a procoagulant state through upregulation of TF-mediated thrombin production, and thrombin itself can be immunogenic in stimulating immune cells to secrete inflammatory mediators (29, 30). Hence, the SNS can influence coagulation via multiple pathways, both directly and indirectly. However, there is currently limited understanding of the interrelationships between the SNS, endotheliopathy, inflammation, and coagulation in the acute period after TBI.

Divergent findings in TBI-related coagulopathy have arisen, in part, from the routine use of *ex-vivo* clotting tests to assess coagulation abnormalities. The international normalized ratio (INR), activated partial thromboplastin time (aPTT), and platelet count (PLT) are the most commonly used diagnostic screens to identify coagulopathy (6, 31, 32). However, these conventional tests measure independent features of the clotting process, do not assess the termination phase of coagulation, and are unable to identify hypercoagulation (12–14). In view of this, blood biomarkers can be informative in developing our understanding of secondary injury cascades after TBI (28, 33), and several candidate markers, such as D-dimers (DD), syndecan-1 (SDC-1), and thrombomodulin (TM), have shown potential in characterizing posttraumatic coagulopathy (34–36). Thus, a comprehensive assessment of biological mediators of coagulopathy and endotheliopathy may improve our understanding of the multifactorial hemostatic responses to injury (37).

The purpose of this study was to characterize peripheral blood biomarkers of coagulopathy and endotheliopathy acutely after isolated TBI, according to 6-month patient outcomes, using multivariate partial least-squares (PLS) analysis. Furthermore, we sought to evaluate the potential covariance between these markers and SNS correlates to provide evidence to support or refute sympathoadrenal hyperactivity as a potential mechanistic driver of coagulopathy.

PATIENTS AND METHODS

Study population and design

As part of a larger prospective observational cohort study (38), this *a priori* subgroup analysis enrolled 159 adult patients with newly acquired TBI at three Level-1 Trauma Centers, from November 2011 to September 2013. Patients were included in the study according to conventional criteria for isolated blunt moderate-to-severe TBI, defined by a Glasgow Coma Scale (GCS) score less than 13 and a nonhead Abbreviated Injury Scale (AIS) score no greater than 2. For complete patient recruitment and clinical data collected, please see our previous works (28, 39). The study was approved by the local Research Ethics Committees and Institutional Review Boards of all participating institutions. All patients or legal representatives were informed of the study details and provided their consent. A group ($n = 27$) of healthy donors free from any medications and without a history of brain injury were included as a control reference in all measurements. All study procedures were conducted in accordance with the declaration of Helsinki including current revisions and Good Clinical Practice guidelines.

Blood sample collection and processing

Venous blood samples were drawn from each patient as soon as possible after admission to the emergency department and again at 24 h postinjury. Specimens for soluble biomarker analyses were obtained from patients and controls using an evacuated tube collection system containing K_2-ethylenediaminetetraacetic acid, lithium-heparin, and Na_3-citrate anticoagulants (BD-Vacutainer; Becton Dickinson, Rutherford, NJ). Anticoagulated blood was centrifuged immediately after sample collection for 20 min at 2,000*g* to obtain platelet-poor plasma, after which the plasma was separated into aliquots and frozen at −80°C until further analysis.

Hemostatic and endothelial marker analysis

Plasma coagulation and fibrinolytic biomarkers were analyzed in duplicate using commercially available IMUBIND quantitative enzyme-linked immunosorbent assay (ELISA) kits (Sekisui Diagnostics, LLC, Lexington, Mass) for TF, tissue factor pathway inhibitor (TFPI), thrombin-activatable fibrinolysis inhibitor (TAFI), thrombin-antithrombin complexes (TAT), plasminogen activator inhibitor-1 (PAI-1), tissue plasminogen activator (tPA), and DD. Soluble endothelial-derived biomarkers SDC-1 and vascular adhesion protein-1 (VAP-1) were analyzed by quantitative ELISA (BioVendor, LLC, Asheville, NC). Absorbencies for all plates were read using an automated microplate photometer (Synergy 2 Multi-Mode Reader with Gen5 Software; BIO-TEK Instruments, Winooski, Vt). All test samples were analyzed in duplicates according to the manufacturer's instructions.

Cytokine, chemokine, and vascular injury marker analysis

Plasma concentrations (pg/mL) of selected cytokines, chemokines, and vascular molecules were analyzed batchwise using Meso Scale Discovery (MSD) 96-Well MULTI-ARRAY/-SPOT Ultra-Sensitive Human Immunoassay Kits: TH1/TH2 10-plex for IFN-γ, IL-1β, -2, -4, -5, -10, -12p70, -13, and TNF-α; Chemokine 9-Plex for Eotaxin, Eotaxin-3, MIP-1β, TARC, IP-10, IL-8, MCP-1, MDC, MCP-4; Vascular Injury 1 and 2 for TM, ICAM-3, E-Selectin, P-Selectin, SAA, CRP, VCAM-1, and ICAM-1. Analytes were detected by electrochemiluminescence on an MSD Sector Imager 6000 (Gaitherburg, Md). All assays were performed in duplicate according to manufacturer's instructions.

Catecholamine analysis

Plasma catecholamine (epinephrine [Epi] and norepinephrine [NE]) concentrations (pmol/L) were determined from duplicate samples using a competitive ELISA method according to the manufacturer's instructions (Bi-CAT EIA, ALPCO Diagnostics, Salem, NH). Absorbance was measured using a multidetection microplate reader (PerkinElmer VICTOR 3, Waltham, Mass).

Statistical analyses

Clinical, demographic, and laboratory variables were dichotomized according to 6-month Extended Glasgow Outcome Scale (GOSE) (favorable, GOSE 5–8; unfavorable, GOSE 1–4) and statistically compared by chi-square, independent Student *t* test, or Mann-Whitney *U*, where applicable. Coagulopathy was calculated using standard laboratory measures INR, aPTT, and PLT. Patients were considered coagulopathic if they had an INR more than 1.3 or aPTT more than 38 s or PLT less than 100,000/μL (10). Multivariate PLS analysis was used to characterize the relationships between blood biomarkers and clinical outcomes. PLS is a supervised technique which identifies optimal combinations of predictor variables that co-vary with either binary (termed PLS-discriminant analysis [PLS-DA]) or continuous response variables (40). A PLS-DA output provides model prediction accuracy (accur) and posterior probability (pprob). Briefly, these indices measure how accurately a fitted model can predict a binary outcome based solely on predictor variables. Accur is evaluated by assigning each subject to the outcome group with the most similar mean PLS score: 1 = correctly predicted and 0 = incorrectly predicted. This provides a simple, robust metric of prediction, which does not depend on a specific probability model. PProb is the likelihood of the PLS model identifying the correct outcome conditional on the observed subject scores under a Gaussian noise model (41). This provides an alternative probabilistic measure that accounts for uncertainty in the PLS model and observed data. When the response variables are continuous, a PLS regression output provides the fraction of variance. Fraction of variance reflects the proportion of total intersubject variability in biomarker data that are described by the PLS component of interest. A high fraction of variance for a single component indicates that subject variability is "one-dimensional," and well described by a single latent variable. Conversely, a low fraction of variance indicates more complex intersubject differences, and cannot be fully captured within a single PLS component. First, PLS-DA analysis was used to identify significant biomarkers in discriminating unfavorable versus favorable patient outcome. Previous research has identified that age and injury severity are associated with patient outcome after TBI (8, 10, 32). Therefore, to account for the influence of these factors, we included age, Injury Severity Score (ISS), AIS head, and GCS in our models. Second, to assess the role of the SNS in mediating acute pathophysiology, PLS was used to identify the covariance between SNS indices (NE and Epi) and biomarkers of coagulopathy and endotheliopathy. All variables were imputed for missing data using the k-means nearest-neighbor method (42) and rank-transformed to ensure robustness against non-normality in biomarker values. Significant variable loadings were derived by performing bootstrap resampling on subjects (1,000 iterations) to obtain empirical *P* values, which were subsequently corrected for multiple comparisons at a false discovery rate (FDR) = 0.05. For all plots, variable loadings are represented as bootstrap ratios (i.e., the bootstrapped mean/SE), which are z-scored statistics reflecting the reliability of variable contributions. Descriptive and univariate statistics were completed using Stata Version 14.1 (StataCorp, College Station, Tex), and multivariate statistics were analyzed using in-house software developed for Matlab, Version R2015b (Matworks, Natick, Mass). Graphs were prepared using GraphPad Prism Version 6.0 h (GraphPad Inc, La Jolla, Calif).

RESULTS

Demographics and clinical characteristics

Patient demographic, clinical, and laboratory variables were dichotomized according to unfavorable and favorable 6-month neurological outcome and shown in Table 1. A total of 159 patients were included in the study, the majority (n = 100; 62.9%) of which had an unfavorable GOSE (1–4) prognosis at 6 months postinjury. Forty-four (27.7%) patients died, 61.4% of these by neurologic death and 38.6% by non-neurologic organ failure. The median time to death was 4 days (range = 1–96 days) (data not shown). The study sample was predominantly male (n = 118; 74.2%), with a mean age of

TABLE 1. **Demographic and clinical characteristics of TBI patients**

Characteristic	All patients (n = 159)	Six-month neurological outcome		*P*
		Favorable (GOSE ≥ 5) (n = 59)	Unfavorable (GOSE < 5) (n = 100)	
Demographics				
Age (years)	45.8 ± 20.3	36.2 ± 15.7	51.5 ± 20.6	**<0.001**
Male sex, n (%)	118 (74.2)	47 (79.7)	71 (71.0)	0.228
Clinical variables				
Time to ED (min)	77.8 ± 63.9	85.9 ± 67.4	73.0 ± 61.6	0.195
ISS score	24.3 ± 11.2	18.6 ± 9.6	27.8 ± 10.7	**<0.001**
Head AIS	4.1 ± 1.1	3.6 ± 1.2	4.4 ± 0.9	**<0.001**
GCS	5.9 ± 2.9	7.2 ± 3.0	5.1 ± 2.6	**<0.001**
Marshall score	2.7 ± 1.3	2.1 ± 1.2	3.0 ± 1.3	**<0.001**
Preinjury comorbidities, n (%)	40 (25.2)	13 (22.0)	27 (27.0)	0.486
Neurosurgey performed, n (%)	46 (28.9)	10 (16.9)	36 (36.0)	**0.003**
Laboratory variables, median (IQR)				
Temperature (°C)	36.0 (35.3–37.0)	36.2 (35.4–37.0)	36.0 (35.0–36.5)	0.300
pH	7.3 (7.3–7.4)	7.3 (7.3–7.4)	7.3 (7.3–7.4)	0.767
SBP (mmHg)	134.0 (120–160)	130.0 (120.0–155.0)	135.5 (120.0–162.0)	0.71
INR (normal <1.3)	1.0 (1.0–1.1)	1.0 (1.0–1.1)	1.1 (1.0–1.2)	**0.001**
aPTT (normal <38 s)	28.0 (25.3–31.4)	27.7 (25.0–29.4)	28.4 (25.8–33.3)	0.08
PLT (normal >100)	218.0 (173.0–266.0)	238.0 (201.0–278.0)	196.0 (166.0–243.0)	**<0.001**
Coagulopathy,* n (%)	20 (19.0)	0 (0.0)	20 (32.3)	**<0.001**
Outcome				
Mortality, n (%)	44 (27.7)	—	—	—
Neurologic, n (% of mortality)	27 (61.4)	—	—	—
Organ failure, n (% of mortality)	17 (38.6)	—	—	—

*Patients were considered coagulopathic if they had admission INR scores more than 1.3 or aPTT more than 38 s or PLT less than 100,000/μL. Coagulopathy data were available on a subset of 105 patients, and percentages were calculated from this number.

AIS, Abbreviated Injury Scale; aPTT, activated partial thromboplastin time; ED, emergency department; GCS, Glasgow Coma Scale; GOSE, Extended Glasgow Outcome Scale; INR, international normalized ratio; IQR, interquartile range; ISS, Injury Severity Score; PLT, platelet; SBP, systolic blood pressure; TBI, traumatic brain injury.

Results displayed as the mean ± SD, unless otherwise stated. Unfavorable versus favorable outcome was computed for each characteristic by Mann-Whitney *U* or chi-square, where appropriate. Significance was determined at $P < 0.05$, and displayed in bold.

45.8 ± 20.3 years. Unfavorable outcome was associated with increased age and injury severity (ISS, AIS head, GCS, Marshall score). Coagulopathy, as defined by INR more than 1.3 or aPTT more than 38 s or PLT less than 100,000/μL, was present in 19.0% of patients at admission, all of which had an unfavorable 6-month outcome. At 24 h, the percentage of patients who were coagulopathic increased to 36.2% (data not shown). Individually, aPTT did not differ in patients according to outcome.

Relationship between biomarkers and 6-month GOSE

Figure 1 depicts the PLS-DA analysis, indicating the combination of biomarkers that optimally distinguished favorable and unfavorable 6-month outcome. For TBI patient blood biomarker concentrations please see Supplementary Table 1, http://links.lww.com/SHK/A418. Variable loadings represent the direction and magnitude of the biomarkers associated with unfavorable outcome. At admission, mean pprob of correct classification of outcome was 0.71, and the mean classification accur was 0.80. In addition to injury severity and age, elevations in 13 blood biomarker variables were significantly associated with unfavorable outcome at FDR = 0.05. The most salient marker at admission was TAT, followed by SDC-1, NE, and tPA, which had higher loading scores than all demographic and clinical indices (Fig. 1A). At 24 h, the pprob and accur were 0.67 and 0.75, respectively; 15 blood biomarkers were significantly associated with unfavorable outcome at FDR = 0.05 (Fig. 1B). Similar to admission, biomarkers with the highest loading scores were SDC-1, tPA, and NE. Contrary to admission, TM, P-Selectin, IL-10, and MDC (negative loading) were significant contributors to outcome at 24 h, although Epi and DD were no longer statistically significant at this time (Fig. 1B).

Covariance between catecholamines and biomarkers

A PLS analysis of the covariance between catecholamine levels (Epi and NE) and blood correlates of endotheliopathy, coagulopathy, and inflammation is shown in Figure 2. At admission, the fraction of variance explained for the relationship between catecholamines and blood biomarkers was 0.81. Fourteen biomarkers showed significant covariation with catecholamine levels at FDR = 0.05. The strongest associations were TAT, followed by TF, SDC-1, and tPA. At 24 h, the fraction of variance explained was 0.74, and 14 biomarkers significantly covaried with Epi and NE. The strongest associations were IL-10, SDC-1, TM, and IL-6 (Fig. 2A). At 24 h, NE displayed a stronger covariant relationship with the predictor biomarkers compared to Epi. Similar to admission, increases in NE and Epi covaried with increases in SDC-1, VAP-1, TAT, TF, IL-6, IL-10, and IL-8. Contrary to admission, increases in E-Selectin, P-Selectin, and TM significantly covaried with Epi and NE (Fig. 2B).

DISCUSSION

This study characterized blood biomarker profiles of coagulopathy and endotheliopathy after isolated TBI, and identified potential mechanistic links between sympathoadrenal activation and these pathologies using multivariate PLS analysis. There were two main findings: unfavorable patient outcome after isolated blunt TBI was associated with acute elevations in circulating biomarkers of endotheliopathy and coagulopathy, and biomarkers of coagulopathy and endotheliopathy co-varied with indices of SNS activity. We evaluated patient biomarker profiles according to 6-month GOSE to accurately identify the biological correlates of coagulopathy that were most influential in determining poor outcome. This is in contrast to patient stratification by ''coagulopathic'' or ''noncoagulopathic'' groups according to standard laboratory tests of coagulopathy; these assays are inherently designed to assess the hypocoagulable state only (12, 13, 43), and may have skewed our results through the selective bias of patients with the greatest hypocoagulable profiles.

Our results are consistent with others who have correlated TBI with an immediate procoagulant, hyperfibrinolytic state (23, 44). At hospital admission, we found the procoagulant markers TAT and TF, and the hyperfibrinolysis marker tPA strongly contributed to unfavorable outcome, whereas the anticoagulant indices TFPI and TM were insignificant. Although levels of TAT and TF remained significantly altered at 24 h, their relative contributions to poor outcome were overshadowed by TM. This change may be supportive of the ''DIC with a hyperfibrinolysis phenotype'' hypothesis, which suggests an early consumption of clotting factors portends a later hypocoagulable state (14, 22, 45). Furthermore, that the two strongest coagulopathic predictors of poor outcome at 24 h were TM and tPA—both important components of the activated protein C pathway (14, 24)—also suggests the potential involvement of this mechanism in a progressive bleeding phenotype. However, it is important to note that the predominant biological function of soluble TM is not well defined; beyond its anticoagulant effects, it is also a well-known marker of endothelial damage (36, 46). Hence, we are unable to definitively conclude that the biomarker phenotype shifted from pro- to anticoagulant in patients over 24 h. In addition, SDC-1, a key proteoglycan marker of endothelial glycocalyx degradation found elevated in major trauma (25, 47), sepsis (35), and acute myocardial infarction (48), was a strong contributor to poor patient outcome at both admission and 24 h. Although these results are supportive of glycocalyx degradation acutely after trauma, it is again difficult to interpret these results in terms of their contributory effects on hypo/hypercoagulation. Shedding of the glycocalyx into the circulation may contribute to a hypocoagulant state through endogenous heparanization (25), although it may also increase circulating concentrations of damage-associated molecular patterns (DAMPs) including hyaluronan fragments and heparin sulfate (49). These DAMPs may then contribute to a hypercoagulant state through the production of inflammatory mediators and subsequent thrombin generation (30); indeed, previous studies have shown glycocalyx disruption increases thrombin production (30, 50). Nevertheless, that SDC-1 strongly co-varied with unfavorable outcome at both admission and 24 h warrants future investigation on the role of the glyocalyx in TBI. Overall, our results are consistent with a relationship between poor outcome and biomarker indices of coagulopathy and endotheliopathy over the first 24 h of hospitalization in isolated TBI patients.

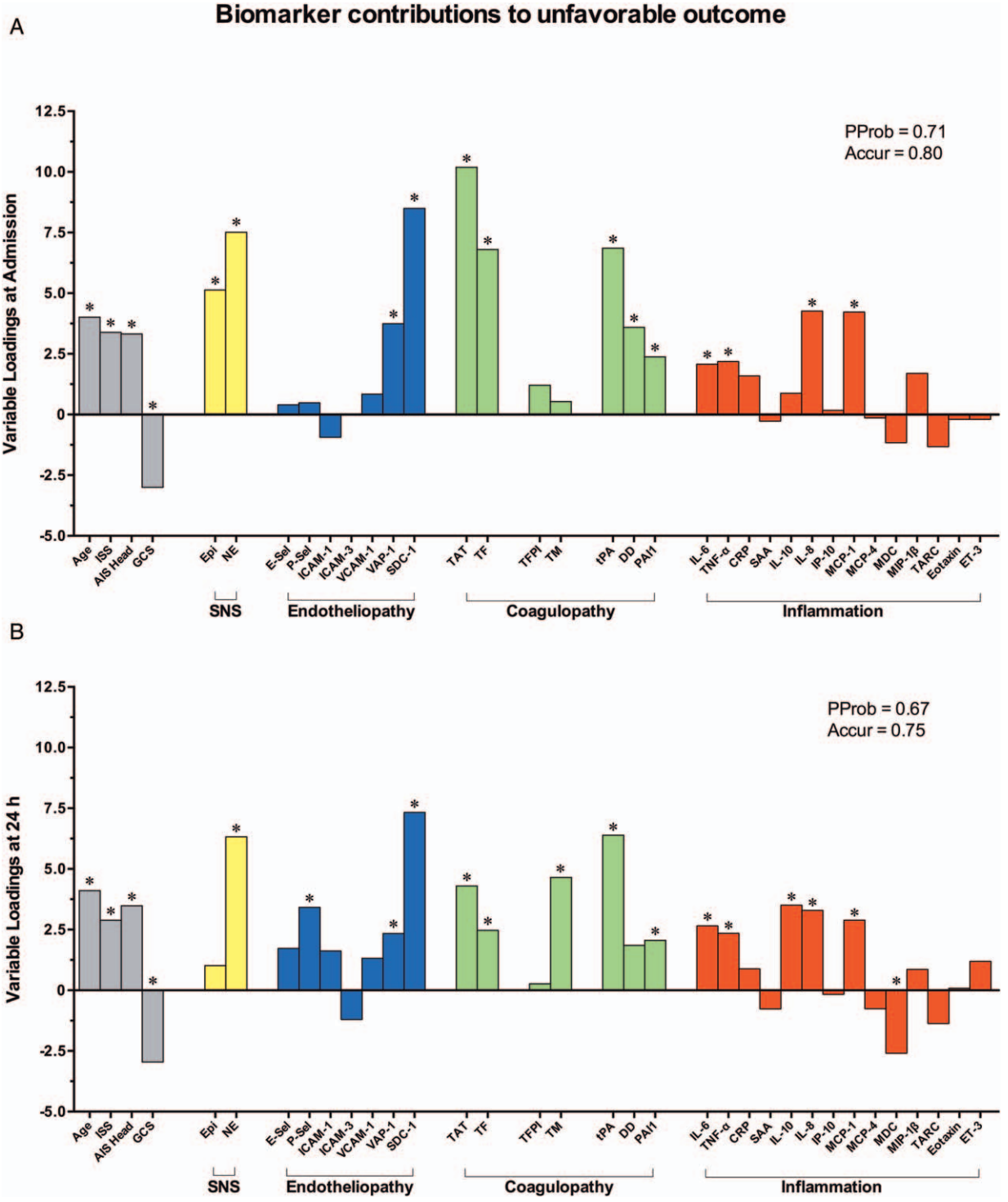

FIG. 1. **Partial least-squares-discriminant analysis of patient outcome.** Biomarker and clinical/demographic predictors discriminating unfavorable from favorable 6-month outcome in TBI patients. Clinical and demographic markers include age, Injury Severity Score (ISS), Head Abbreviated Injury Scale (AIS) score, and Glasgow Coma Scale (GCS) scores. Blood biomarkers include *sympathetic nervous system (SNS) biomarkers*—epinephrine (Epi) and norepinephrine (NE); *endotheliopathy biomarkers*—E-Selectin (E-Sel), P-Selectin (P-Sel), intercellular adhesion molecule (ICAM)-1, -3, vascular cell adhesion molecule (VCAM)-1, vascular activation protein (VAP)-1, syndecan (SDC)-1; *coagulopathy biomarkers*—thrombin-antithrombin complex III (TAT), tissue factor (TF), tissue factor platelet inhibitor (TFPI), thrombomodulin (TM), tissue plasminogen activator (tPA), D-dimer (DD), plasminogen activation inhibitor (PAI)-1; *inflammation biomarkers*—interleukin (IL)-6, tumor necrosis factor (TNF)-α, c-reactive protein (CRP), serum amyloid A (SAA), IL-10, -8, interferon producing protein (IP)-10, monocyte chemoattractant protein (MCP)-1, -4, macrophage-derived chemokine (MDC), macrophage inflammatory protein (MIP)-1β, thymus and activation regulated chemokine (TARC), eotaxin, and eotaxin-3 (ET-3). Blood biomarker contributions are displayed at admission (A) and 24 h (B). Bars represent z-scores derived from individual bootstrapped loadings divided by the SE of the mean. *Significance at FDR = 0.05.

Evidence suggests SNS hyperactivity as a likely mechanistic driver of posttrauma coagulopathy (51–53). In the current study, 81% of the variance observed at hospital admission in SNS correlates NE and Epi was explained by variance in biomarkers of coagulopathy and endotheliopathy. The strongest covariate was TAT, followed by TF, SDC-1, and markers of

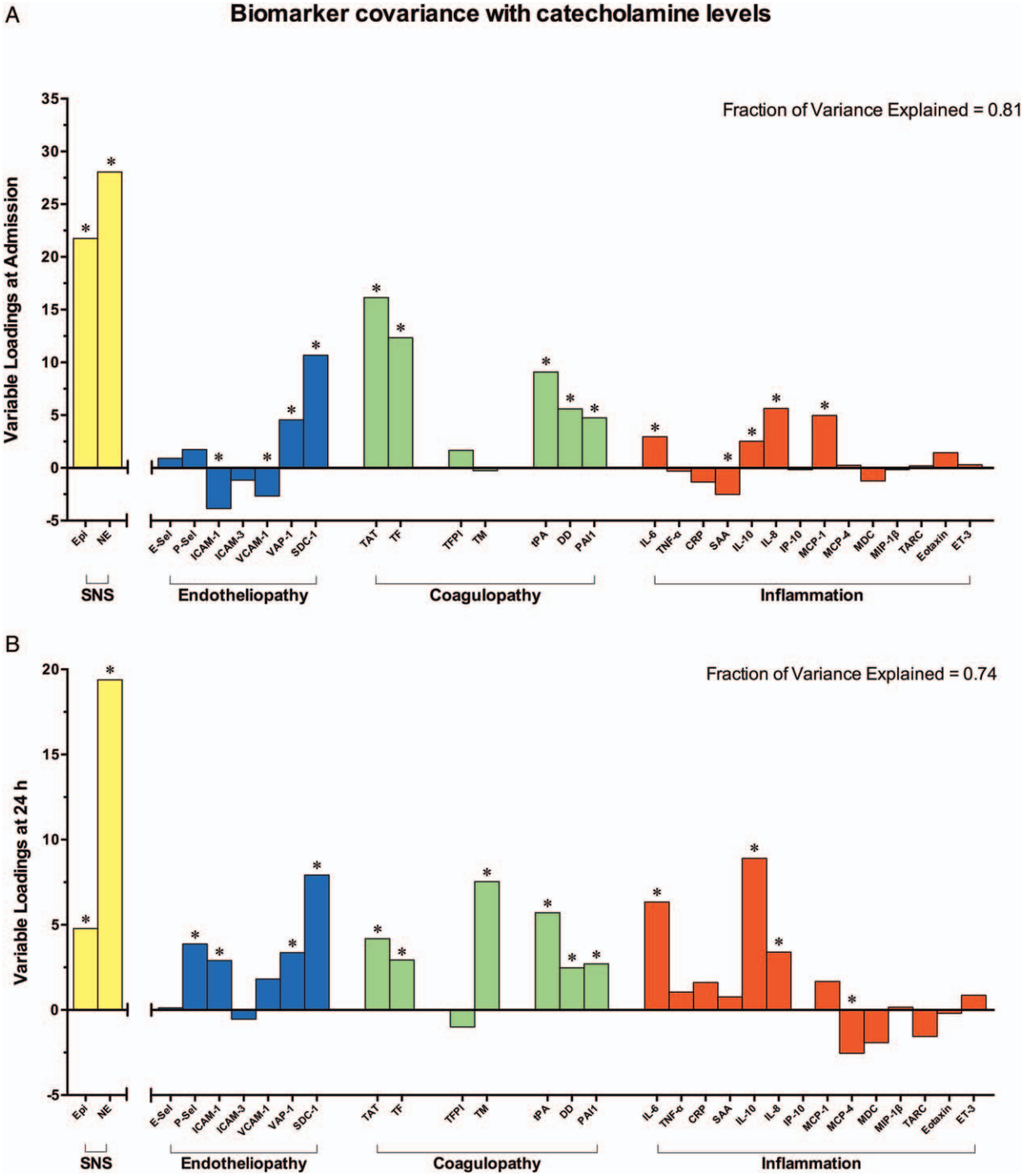

FIG. 2. **Partial least-squares analysis of covariance between the SNS and markers of endotheliopathy, coagulopathy, and inflammation.** *SNS biomarkers*—epinephrine (Epi) and norepinephrine (NE); *endotheliopathy biomarkers*—E-Selectin (E-Sel), P-Selectin (P-Sel), intercellular adhesion molecule (ICAM)-1, -3, vascular cell adhesion molecule (VCAM)-1, vascular activation protein (VAP)-1, syndecan (SDC)-1; *coagulopathy biomarkers*—thrombin-antithrombin complex (TAT), tissue factor (TF), tissue factor platelet inhibitor (TFPI), thrombomodulin (TM), tissue plasminogen activator (tPA), D-dimer (DD), plasminogen activation inhibitor (PAI)-1; *inflammation biomarkers*—interleukin (IL)-6, tumor necrosis factor (TNF)-α, c-reactive protein (CRP), serum amyloid A (SAA), IL-10, -8, interferon producing protein (IP)-10, monocyte chemoattractant protein (MCP)-1, -4, macrophage-derived chemokine (MDC), macrophage inflammatory protein (MIP)-1β, thymus and activation regulated chemokine (TARC), eotaxin, and eotaxin-3 (ET-3). Blood biomarker contributions are displayed at admission (A) and 24 h (B). Bars represent z-scores derived from individual bootstrapped loadings divided by the SE of the mean. *Significance at FDR = 0.05.

hyperfibrinolysis (tPA, DD). The observed change in biomarker profiles toward an anticoagulant state at 24 h was mirrored in our covariation analysis; at 24 h the explained variance was 74%, NE became the strongest catecholamine covariate, and was highly associated with increases in SDC-1, TM, and tPA. Indeed, Ostrowski et al. have produced a number of studies correlating sympathoadrenal activation to endothelial damage and indices of hyperfibrinolysis, and have identified an interrelationship between catecholamines and glycocalyx injury in multiple trauma modalities (25–27, 34, 35, 48, 54). That we

found covariance between Epi, NE and correlates of glycocalyx damage (SDC-1), endothelial activation (E-Selectin, VAP-1), and hyperfibrinolysis (tPA, DD), is consistent with the works by this group and further suggests similarities in SNS-mediated endotheliopathic and hyperfibrinolytic mechanisms in multisystem trauma and isolated TBI.

A complex, bidirectional relationship exists between SNS activity and both inflammation (28, 55, 56) and coagulopathy (29, 57). In the current study, poor outcome and heightened SNS activity co-varied with a procoagulant/proinflammatory biomarker phenotype on hospital admission that progressed toward an anti-inflammatory phenotype at 24 h postinjury due to the increased contribution of IL-10. In support of this, it has been shown that chemical sympathectomy diminishes inflammation, glycocalyx shedding, and coagulopathy in rats (56). In addition, proinflammatory cytokines are capable of inducing coagulation through their ability to generate TF (29, 57), and it follows that increases in counterregulatory anti-inflammatory mediators may contribute to a progressive shift from a pro- to anticoagulative state. However, as coagulopathic mediators such as thrombin and TM may also induce pro- and anti-inflammatory responses, respectively (51, 58), the directionality of the relationship between coagulopathy and inflammation remains uncertain. Furthermore, it is possible that the covariance we observed between catecholamines and biomarkers of inflammation, coagulopathy, and endotheliopathy was influenced by their mutual relationship to injury severity. However, we found that biomarkers co-varied with catecholamines to a greater extent than with indices of injury severity (fraction of variance explained)—Epi, NE: 81% at admission, 74% at 24 h, vs. GCS, AIS head, ISS: 67% at admission, 68% at 24 h (Figure, Supplemental Digital Content 2, at http://links.lww.com/SHK/A398). This finding, together with our outcome analysis which showed that individual biomarker contributions to unfavorable outcome often exceeded the contributions of traditional indices of injury severity (Fig. 1), is consistent with a pathological relationship between SNS activation and coagulopathy, endotheliopathy, and inflammation.

There were several limitations of the current study. Despite evaluating multiple biomarkers spanning different facets of coagulopathy in a well-controlled clinical design, the analysis of additional cofactors may have been helpful to fully assess hemostatic alterations. However, our study design was proscribed in terms of preanalytical considerations and on the basis of available patients' sample volume. Likewise, we were only able to assess the selected biomarkers at two time points (i.e., at admission and 24 h postinjury), whereas a greater sampling frequency may have been valuable in evaluating potentially rapid kinetic changes in the acute biomarker profiles. Moreover, the ability to correlate our biomarker data with additional clinical laboratory indices such as thromboelastometry—capable of identifying hypercoagulable states—could have strengthened our findings. Furthermore, although our cohort was representative of the general trauma population regarding sex distribution, our sample size prevented the assessment of biomarker profiles between males and females. Indeed, previous studies have shown that trauma can elicit sex-specific immune and coagulopathic responses; females may be immunologically protected through the effects of the sex steroid 17β-oestradial (59), whereas females with acute trauma coagulopathy may have worse outcomes compared with males (60). Nonetheless, our results provide a novel in-depth assessment of coagulopathy in a large cohort of isolated TBI patients, and are consistent with the notion that this process is related to poor outcome and is mediated by sympathoadrenal hyperactivity.

In conclusion, biomarkers of coagulopathy and endotheliopathy are associated with poor outcome in isolated TBI patients. Patients with poor outcome exhibit increased circulating markers of glyocalyx and endothelial damage, vascular activation, inflammation, procoagulation, and hyperfibrinolysis. Moreover, SNS activity as assessed by circulating catecholamines is highly correlated with markers of endotheliopathy and coagulopathy within the first 24 h after injury. Additional research is warranted to further characterize the pathogenic role of sympathoadrenal-mediated hemostatic alterations in isolated TBI.

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