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CIRCULATING SYNDECAN-1 AND TISSUE FACTOR PATHWAY INHIBITOR, BIOMARKERS OF ENDOTHELIAL DYSFUNCTION, PREDICT MORTALITY IN BURN PATIENTS

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ABSTRACT—**Objective:** The aim of this study is to evaluate the association between burn injury and admission plasma levels of Syndecan-1 (SDC-1) and Tissue Factor Pathway Inhibitor (TFPI), and their ability to predict 30-day mortality. **Background:** SDC-1 and TFPI are expressed by vascular endothelium and shed into the plasma as biomarkers of endothelial damage. Admission plasma biomarker levels have been associated with morbidity and mortality in trauma patients, but this has not been well characterized in burn patients. Methods: This cohort study enrolled burn patients admitted to a regional burn center between 2013 and 2017. Blood samples were collected within 4 h of admission and plasma SDC-1 and TFPI were quantified by ELISA. Demographics and injury characteristics were collected prospectively. The primary outcome was 30-day in-hospital mortality. **Results:** Of 158 patients, 74 met inclusion criteria. Most patients were male with median age of 41.5 years and burn TBSA of 20.5%. The overall mortality rate was 20.3%. Admission SDC-1 and TFPI were significantly higher among deceased patients. Plasma SDC-1 >34 ng/mL was associated with a 32-times higher likelihood of mortality [OR: 32.65 (95% CI, 2.67–399.78); $P = 0.006$] and a strong predictor of mortality (area under the ROC [AUROC] 0.92). TFPI was associated with a nine-times higher likelihood of mortality [OR: 9.59 (95% CI, 1.02–89.75); $P = 0.002$] and a fair predictor of mortality (AUROC 0.68). **Conclusions:** SDC-1 and TFPI are associated with a higher risk of 30-day mortality. We propose the measurement of SDC-1 on admission to identify burn patients at high risk of mortality. However, further investigation with a larger sample size is warranted.

KEYWORDS—Burn, CD-138, endotheliopathy, glycocalyx, SDC-1, TFPI

INTRODUCTION

The effect of shock states on the structure and function of the endothelium is a rapidly growing area of surgical research. Structural damage to the endothelium and its physiologic consequences have been broadly defined as endotheliopathy. Endotheliopathy is a complex process that can be initiated by tissue damage, inflammation, shock, and ischemia and leads to poor outcomes for critically ill patients (1–4). The concept of shock-induced endotheliopathy has been described in several settings including hemorrhagic, septic, and cardiogenic shock (4–6).

The endothelial glycocalyx layer (EGL) is a complex network of proteoglycans and glycoproteins on the luminal surface of blood vessels that maintains vessel integrity and regulates inflammation, blood clotting, and flow (7, 8). Proteolytic release of EGL components (shedding) occurs in response to a number of physiologic challenges and results in detrimental systemic effects (9). For example, acute endogenous autoheparinization has been linked to endothelial glycocalyx degradation mediated through the release of heparan sulfate bearing proteoglycans (3, 10, 11).

Syndecan-1 (SDC-1), a transmembrane proteoglycan, is a component of the EGL and is substituted with heparan sulfate chains which promote its interactions with key anticoagulants including tissue factor pathway inhibitor (TFPI). SDC-1 is shed into the bloodstream upon endothelial injury and serves as a potential biomarker for endotheliopathy (12). Elevated levels of SDC-1 in trauma patients on admission have been associated with

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inflammation, coagulopathy, and increased mortality (1, 2, 13). In one large prospective observational study of 424 patients who suffered traumatic injury, the authors concluded that SDC-1 was an independent predictor of < 24-h, 7-day, and 28-day mortality (13).

TFPI is a coagulation inhibitor primarily synthesized by endothelial cells. It has a relatively complex distribution in the vasculature, with circulating and surface-associated populations. It has two major isoforms: TFPI-ß is an active, truncated form anchored to the endothelial surface *via* a glycosylphosphatidylinositol moiety; TFPI-α is an active, full-length form found in plasma. In addition, a large reservoir of TFPI-α (two to four times the plasma concentration (14, 15)) is noncovalently bound to endothelial cell heparan sulfate proteoglycans such as SDC-1. Platelets also contain TFPI-α, with the platelet-associated pool amounting to ~10% of the total plasma pool. Both isoforms function to suppress the onset of tissue factor dependent coagulation by reversibly inhibiting both FXa directly and the tissue factor-FVIIa complex (extrinsic tenase) in a FXa-dependent mechanism (16, 17). In plasma, 80% to 90% of TFPI antigen is found associated with lipoproteins, with about 10% circulating free.

Early recognition of endotheliopathy with the use of biomarkers can be useful for clinicians. While advances in understanding of this pathophysiology in blunt and penetrating trauma have been promising, endotheliopathy in burn-injured patients has not been well studied, and the contribution of endothelial dysfunction in burn pathophysiology is largely unknown. There are very few animal or human studies that investigate SDC-1 levels in subjects with primarily thermal injuries (18–20). Osuka et al. (19) found that SDC-1 as a marker of endothelial damage was correlated with age and increased 24-h fluid requirements in burn patients, but not with burn size or mortality. Welling et al. (20) compared burn and non-burn trauma patients with endotheliopathy defined by SDC-1 levels ≥ 40 ng/mL. They found that inhalation injury combined with endotheliopathy led to higher mortality rates in burn patients (20). Previous work by our lab using a mouse model determined that EGL shedding measured by SDC-1 was correlated to burn injury severity (21). Furthermore, in a rat model of burn injury we demonstrate that burn injury induces SDC-1 shedding and plasma-based resuscitation may ameliorate subsequent vascular leakage (22). To our knowledge, there is only one animal study investigating TFPI levels in a rat model of burn injury and sepsis (23). Animal models can be used to determine associations between biomarkers and burn injury, but clinical studies are needed to investigate outcomes. To further the understanding of endotheliopathy in burn injury, this study aims to investigate the relationship between burn injury severity, admission SDC-1, and TFPI levels as biomarkers of endothelial dysfunction, and morbidity and mortality.

METHODS

Study population

This study was approved by the Institutional Review Board of MedStar Health Research Institute (IRB #2012-029) and the Human Research Protection Offices of the Departments of the Navy and Army. All patients over the age of 18 years who presented to a regional burn center within 4 h of thermal injury due to flash, flame, or contact with anticipated need for hospital admission were screened for enrollment. Patients with a pre-existing history of coagulopathy, those taking anticoagulant medications, pregnant women, chemically injured patients, children, and patients not fluent in English or Spanish were excluded. A total of 158 patients were enrolled. For the present analysis, additional inclusion criteria were arrival and admission blood draw within 4 h of injury with available admission SDC-1 and TFPI data. Seventy-four individuals were included in the present analysis (Fig. 1A). Patients were enrolled prospectively into this observational study between 2013 and 2017. Sampling and other procedures have been described in detail elsewhere (24).

Clinical patient data

Patient demographic information, laboratory data, and treatment information were collected prospectively from the medical record. Inhalation Injury was diagnosed based on clinical suspicion and/or bronchoscopic findings.

Sample collection

Baseline admission blood samples were collected from patients within 4 h of the injury into SCAT-144 tubes (500 μM of AEBSF, 20 μM of elastinal, 10 μM of GGACK, 4.5 mM of EDTA, 5 μM of E64, 1 μM of Pepastin A, 300 KIU/mL of aprotinin; Haematologic Technologies, Essex Junction, Vt). Tubes were spun at 400 g for 10 min after which platelet-rich plasma was isolated and respun at 3,000 g for 17 min. Platelet-poor plasma aliquots were flash frozen and stored at −80°C.

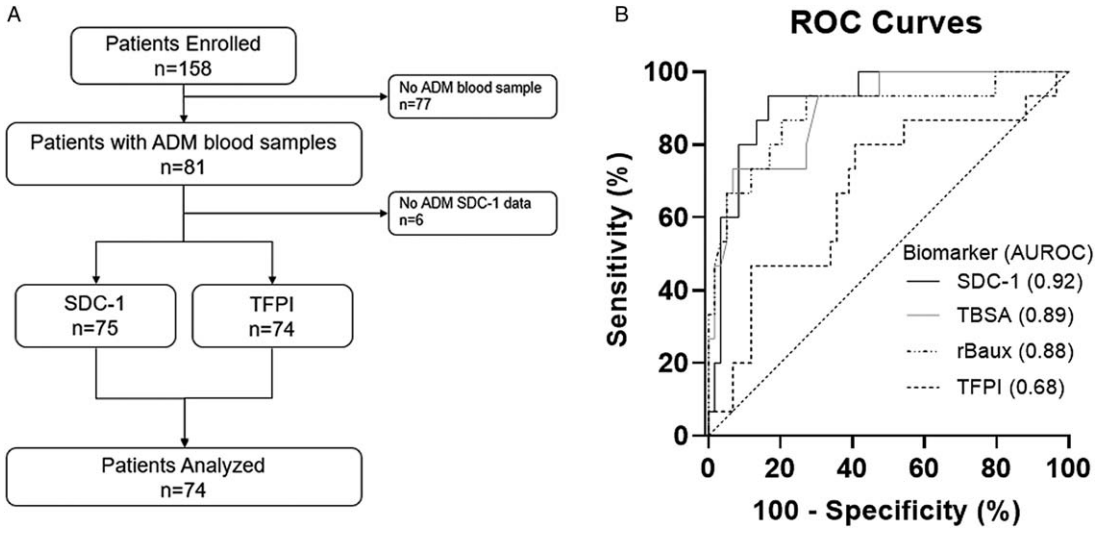

Fig. 1. **Study population (A) and receiver operating characteristic (ROC) curves for SDC-1 and TFPI (B).**

Biomarker measurements

Plasma SDC-1 and TFPI levels were quantified by ELISA. The assays were performed per the protocols from the manufacturers for SDC-1 (Human CD 138: Diaclone SAS, Besancon Cedex, France), and TFPI (Asserachrom Total TFPI: Stago, Parsippany, NJ). Samples were assayed in duplicate at dilutions that resulted in readings within standard curves characterized by maximum absorbance values < 1.5. Inter-assay variability was monitored by assaying the same multidonor plasma pool from healthy individuals, kit controls, and/or in-house standards on each assay plate.

Thromboelastography

Viscoelastic properties of clot formation and lysis were measured using the TEG 5,000 (TEG, Haemonetics, Boston, Mass). The RapidTEG reagent was used to initiate clot formation according to the manufacturer's instructions. RapidTEG (rTEG) activates both the intrinsic and extrinsic clot formation pathways to provide a rapid assessment of clot formation, clot strength, and fibrinolysis. Measured parameters include activated clotting time (ACT), α-angle, maximum amplitude (MA), and clot lysis at 30 min (LY30). These parameters measure the speed of clot initiation, rate of clot development, maximum clot strength, and rate of fibrinolysis respectively. Reference ranges were defined by the manufacturer for α-angle (66°–82°), MA (54 mm–72 mm), and LY30 (0%–8%). An ACT value of >140 s was considered abnormal (25).

Outcomes

The primary outcome measure was 30-day in hospital mortality. Secondary outcomes included morbidities such as intensive care admission, ventilator requirement, and measures of injury severity including burn size, and prehospital or emergency room intubation.

Statistical analysis

Follow-up times were prespecified in the study as 30-days from hospital admission (ADM) to death, discharge, or censoring on the 30th day post-ADM. Burn size was stratified as small if percent total body surface area (TBSA) was ≤10%, moderate if %TBSA was between 10% and 30%, and large if %TBSA ≥30%.

The receiver operating characteristic (ROC) curve was used to discriminate between SDC-1 and TFPI on ADM in predicting 30-day mortality. ROC curves were also created for TBSA and the revised Baux Score (26), to compare SDC-1 and TFPI to existing clinical prognostic tools for burn patient mortality. The Hosmer–Lemeshow goodness-of-fit test was used for calibration of the model between SDC-1, TFPI, TBSA, and the revised Baux score. The Akaike Information Criteria (AIC) was used for model fit. The optimal cutoff values for SDC-1 and TFPI were obtained by ROC curves analysis with Youden index. Each marker was evaluated by its sensitivity, specificity, positive and negative predictive values, and area under the ROC with 95% confidence interval (95% CI).

Admission SDC-1 and TFPI were *a priori* defined as a higher or lower than their optimal cutoff points of 34 ng/mL and 73 ng/mL, respectively. Admission rTEG parameters were compared between patients with admission SDC-1 and TFPI levels above and below these cutoffs. Likelihood of mortality (odds ratios [ORs]) calculated using logistic regression and time to death (hazard ratios [HRs]) computed using the Cox proportional hazards models were used to evaluate the association and effect of ADM SDC-1 and TFPI levels on mortality while adjusting for possible confounding factors. These confounding factors included age, gender, BMI, burn TBSA (%), inhalation injury, transport method, and Glasgow coma scale (GCS) as well as their interactions, selected using the minimum AIC. Kolmogorov-type supremum test was used for the Cox proportional hazards assumption. Kaplan–Meier plots and log-rank tests were used for survival analysis.

Descriptive statistics characterized the demographics and injuries of the patients by survival status. Categorical variables were presented as frequencies and percentages and tested using the chi-square or Fisher exact test. Continuous variables were expressed as means and standard deviations (SDs) or medians and interquartile ranges (IQRs) and tested for differences between defined groups using the *t* test or Mann–Whitney test as appropriate. Statistical significance was determined at the $P < .05$ level (two-sided). All data were analyzed using SAS, version 9.4 (SAS Institute Inc) and GraphPad Prism version 8 for Windows (Graphpad Software, San Diego, Calif).

RESULTS

Demographics

SDC-1 and TFPI levels at ADM were obtained for a total of 74 patients (Fig. 1A). Demographics and injury characteristics are presented in Table 1. Most patients were male (75.7%), median age was 41.5 years (IQR, 30–59 years), and median TBSA was 20.5% (IQR, 7%–41%). Overall mortality was 20.3% (n = 15). Non-survivors were older, more likely to arrive by helicopter, with larger %TBSA burns, higher incidence of concomitant inhalation injury, lower GCS, were all intubated emergently, and were more likely to require mechanical ventilation. There was no difference between groups in the proportion of patients who required transfusion; however, a greater proportion of survivors received red blood cells. There was no difference in the proportion of patients requiring operative intervention; however among patients who died, time to operative intervention was shorter. Survivors experienced longer intensive care unit (ICU) and hospital length of stay reflective of ongoing care (Table 1).

Biomarkers of endotheliopathy as predictors of mortality

Admission SDC-1 and TFPI were higher in patients who died than in those who survived, with median (IQR) of 58.0 ng/mL (IQR, 42.3 ng/mL–97.3 ng/mL) versus 20.9 ng/mL (IQR, 14.1 ng/mL–27.9 ng/mL); $P < 0.0001$, and 78.6 ng/mL (73.6 ng/mL–110.2 ng/mL) versus 70 ng/mL (57.9 ng/mL–83.7 ng/mL); $P = 0.04$, respectively (Table 1). There is a weak correlation between AMD SDC-1 and TFPI levels (Rho = 0.33; $P = 0.004$). About 23% of patients (n = 17) had both high SDC-1 (>34 ng/mL) and TFPI (>73 ng/mL) and 41% had both low SDC-1 and TFPI ($P = 0.01$). To identify a cut-off level of SDC-1 and TFPI to predict mortality, we constructed ROC analyses with Youden index using 30-day in-hospital mortality as the outcome variable. For SDC-1 the area under the curve was 0.92 (95% CI 0.85–0.99) (Fig. 1B). The cutoff (SDC-1 >34 ng/mL) was determined by the ADM plasma level of SDC-1 that maximized the sum of sensitivity and specificity in predicting 30-day in-hospital mortality (sensitivity (SN) 93%, specificity (SP) 83%, positive predictive value (PPV) 58.3%, negative predictive value (NPV) 98%). Higher SDC-1 at ADM was associated with a higher risk of mortality (Fig. 2A). For TFPI the area under the curve was 0.68 (95% CI 0.51–0.86) (Fig. 1B). The TFPI cutoff (TFPI > 73 ng/mL) was determined as above (SN 81%, SP 59%, PPV 35.1%, NPV 92.1%). Higher TFPI at ADM was associated with a higher risk of mortality (Fig. 2B). The performance of SDC-1 as a predictor of 30-day in-hospital mortality was superior to TFPI ($P = 0.007$, Fig. 1B) and the Hosmer–Lemeshow goodness-of-fit test used for calibration of the models confirmed this after adjusting for possible confounding factors. SDC-1 performed similar to the revised Baux score and TBSA in predicting mortality ($P > 0.05$, Fig. 1B).

Thromboelastography

Median admission rTEG parameters in all groups fell within reference ranges for ACT, α-angle, MA, and LY30. There were no significant differences between patients with low (n = 27) and high (n = 18) SDC-1 on ACT (124.5 s, 113–136 s vs. 113 s, 113–167 s; $P = 0.84$), α-angle (74.2°, 69.5°–77.2° vs. 74.6°, 53.8°–76.1°; $P = 0.24$), MA (62.4 mm, 58.3 mm–65.3 mm vs. 61.5 mm, 42.5 mm–64.3 mm; $P = 0.40$), or LY30 (1.1%, 0%–2.1% vs. 0.9%, 0%–1.8%; $P = 0.98$). There were no significant

TABLE 1. **Demographics and injury characteristics**

Characteristic	All	Mortality		*P* value
		No	Yes	
No. of patients, No. (%)	74 (100.0)	59 (79.7)	15 (20.3)	–
Male, No. (%)	56 (75.7)	44 (74.5)	12 (80.0)	0.99
Age, year	41.5 (30.0–59.0)	37.0 (28.0–52.0)	60.0 (54.0–68.0)	0.0002
Ethnicity, No. (%)				0.13
Caucasian	25 (33.8)	19 (32.0)	6 (40.0)	
African American	26 (35.1)	22 (37.3)	4 (26.7)	
Hispanic	10 (16.9)	10 (13.5)	0 (0.00)	
Other	13 (17.6)	8 (13.6)	5 (33.3)	
BMI, median (IQR)	26.7 (23.8–30.2)	27.0 (23.7–30.9)	26.4 (24.2–29.9)	0.75
Transport method, No. (%)				0.04
Helicopter	32 (43.2)	22 (37.3)	10 (66.7)	
Ambulance	42 (56.8)	37 (62.7)	5 (33.3)	
Time POI to ADM, min	55 (42–83)	55 (42–96)	54 (42–75)	0.67
Time ADM to blood draw, min	49 (23–87)	47 (23–84)	51 (21–96)	0.63
Time POI to blood draw, min	112 (85–170)	112 (85–170)	130 (75–175)	0.95
Total %TBSA burned, %	20.5 (7.0–41.0)	11.5 (4.0–32.0)	55.0 (26.5–93.0)	<0.0001
TBSA ≤10%, No. (%)	27 (36.5)	27 (45.8)	0 (0.0)	0.001
>10% and <30%	20 (27.0)	16 (27.1)	4 (26.7)	
≥30%	27 (36.5)	16 (27.1)	11 (73.3)	
Inhalation injury, No. (%)	21 (28.4)	13 (22.0)	8 (53.3)	0.02
Polytrauma, No, (%)	9 (12.2)	8 (13.6)	1 (6.7)	0.68
GCS at ADM,	15 (9–15)	15 (14–15)	3 (3–15)	0.0004
Intubation PTA/ADM, No. (%)	41 (55.4)	26 (44.1)	15 (100)	0.0001
LOS days	10 (2–30)	12 (3–54)	2 (1–12)	0.002
ICU, yes, No. (%)	48 (64.9)	36 (61.0)	12 (80.0)	0.17
ICU LOS, median (IQR)	13 (2–30)	18 (4–47)	2 (1–13)	0.01
Ventilated, No. (%)	34 (46.6)	20 (34.5)	14 (93.3)	<0.0001
Days on ventilator, median (IQR)	9 (1–23)	22 (5–35)	2 (1–6)	0.002
Blood Transfusion, yes, No. (%)	30 (40.5)	22 (37)	8 (53)	0.26
PRBC, Yes, No. (%)	24 (80)	20 (91)	4 (50)	0.03
FFP, Yes, No. (%)	26 (86)	18 (82)	8 (100)	0.55
Operation, Yes, No. (%)	49 (66)	42 (71)	7 (47)	0.07
Time ADM to OR, h	39 (22–78)	44 (27–86)	14 (7–36)	0.05
ADM SDC-1	25.2 (16.1–38.3)	20.9 (14.1–27.9)	58.0 (42.3–97.3)	<0.0001
ADM TFPI	73.0 (59.9–91.0)	70.0 (57.9–83.7)	78.6 (73.4–110.2)	0.04

ADM indicates admission; BMI, body mass index; FFP, fresh frozen plasma; GCS, Glasgow Coma Scale; ICU, intensive care unit; LOS, length of stay; OR, operating room; POI, point of injury; PTA, prior to arrival; RBC, packed red blood cells; SDC-1, syndecan-1; TBSA, total body surface area; TFPI, tissue factor pathway inhibitor.

differences between patients with low (n = 20) and high (n = 25) TFPI on ACT (130 s, 105–136 s vs. 113 s, 113–136 s; $P = 0.15$), α-angle (74.3°, 69.3°–76.6° vs. 74.2°, 66°–77.2°; $P = 0.98$), MA (62.6 mm, 57.9 mm–64.7 mm vs. 62.2 mm, 55.0 mm–64.7 mm; $P = 0.96$), or LY30 (1.2%, 0.2%–1.6% vs. 0.7%, 0–2.4%; $P = 0.87$).

Likelihood of mortality and time to death

Likelihood of mortality (ORs) and time to death (HRs) were computed to investigate associations with admission biomarker levels while adjusting for possible confounders (Table 2). On univariate analysis each year increase in age was associated with a higher likelihood of mortality as well as shorter time to death (OR 1.09, HR 1.07, $P = 0.0006$). Increasing %TBSA burn was associated with higher likelihood and shorter time to mortality (OR 1.07, HR 1.05, $P < 0.0001$), and the presence of inhalation injury was associated with a higher likelihood of mortality (OR 4.04, $P = 0.02$). After adjusting for these confounders, a high ADM SDC-1 level (> 34 ng/mL) was associated with a 32 times higher risk for mortality [OR: 32.65 (95% CI, 2.67–399.78); $P = 0.006$] and a 14-fold reduction in the time to death [HR 14.08 (95% CI, 1.70–116.60); $P = 0.01$] (Table 2). A high ADM TFPI level (>73 ng/mL) conveyed nine times higher risk of mortality [OR: 9.59 (95% CI, 1.02–89.75); $P = 0.002$] but did not affect the time to death (Table 2). Kaplan–Meier survival analysis showed as significant difference among patients stratified by an ADM SDC-1 cutoff of >34 ng/mL ($P < 0.0001$) as well as an ADM TFPI cutoff of >73 ng/mL ($P = 0.02$) (Fig. 2, C and D).

Association between biomarkers of endotheliopathy and injury severity, and secondary outcomes

There was a significantly higher proportion of large burns (>30% TBSA) in the deceased group (Table 1). Median admission SDC-1 levels increased in a stepwise progression from small (15.7 ng/mL, 9.4 ng/mL–26.4 ng/mL), to moderate (25.7 ng/mL, 17.2 ng/mL–41.6 ng/mL), to large burns (37.6 ng/mL, 21.3 ng/mL–49.1 ng/mL) ($P = 0.0001$). Median admission TFPI levels also increased from small (64.4 ng/mL, 51.4 ng/mL–80.4 ng/mL), to moderate (72.4 ng/mL, 62 ng/mL–94.7 ng/mL), to large burns (81.9 ng/mL, 64.4 ng/mL–106.2 ng/mL) ($P = 0.03$). Using the established SDC-1 and TFPI cutoffs of 34 ng/mL and 73 ng/mL respectively, we analyzed the proportion of patients by TBSA categories, ICU admission, and ventilator requirement. There was a greater proportion of patients with a higher SDC-1 (>34 ng/mL)

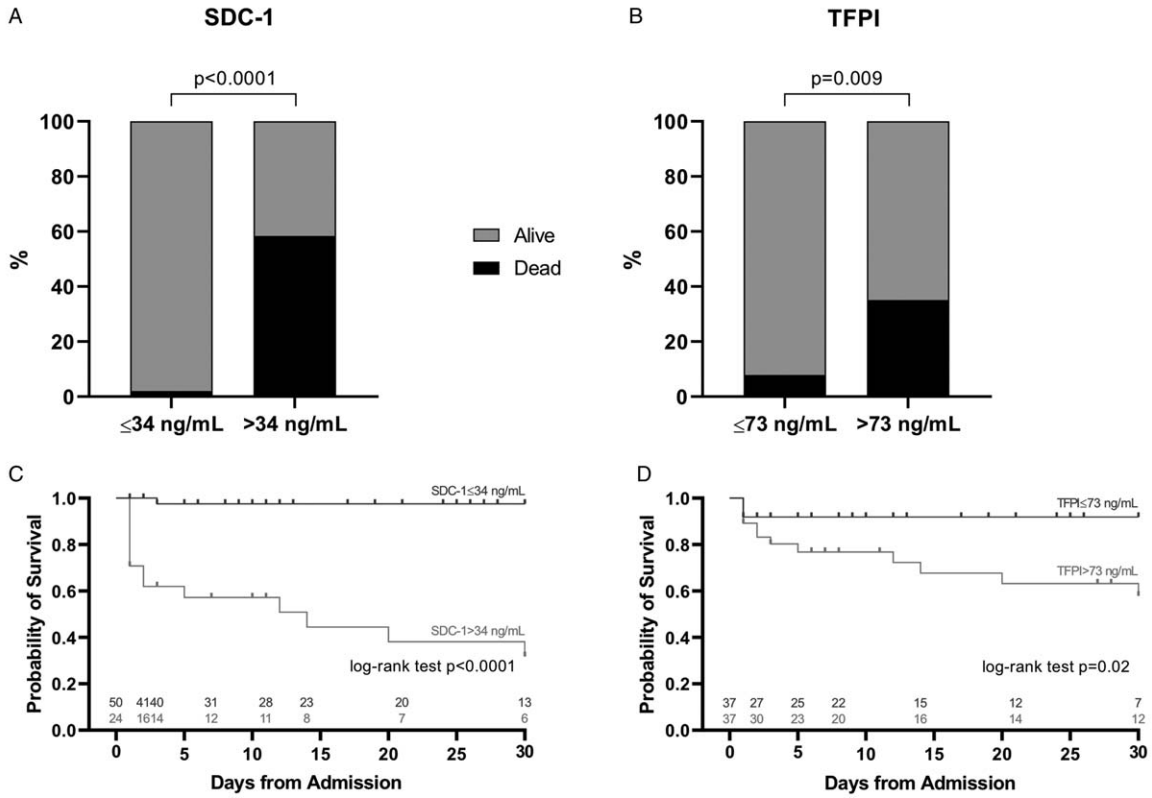

FIG. 2. **Admission SDC-1 and TFPI associated with (OR, A, B) and time to (HR, C, D) mortality based on cut-off levels of 34 ng/mL and 73 ng/mL respectively.** SDC-1 indicates syndecan-1; TFPI, tissue factor pathway inhibitor.

moving from small, to moderate, to large burns (Fig. 3A, $P = 0.002$), there was a similar trend observed for a higher TFPI (>73 ng/mL) (Fig. 3E; $P = 0.16$). There was a greater proportion of patients requiring ICU admission with higher SDC-1 and TFPI levels, but this finding did not reach statistical significance (Fig. 3, B and F). The patients with higher SDC-1 levels were more likely to require mechanical ventilation (Fig. 3C, $P = 0.002$); there was a similar trend with marginal significance for TFPI (Fig. 3G; $P = 0.08$). Patients with higher SDC-1 levels were more likely to require intubation prior to arrival or on ADM (Fig. 3D, $P = 0.0008$), but this was not the case for TFPI (Fig. 3H).

TABLE 2. **Likelihood and time to death**

Univariate	OR (95% CI)	*P* value	HR (95% CI)	*P* value
Sex, female versus male	0.73 (0.18–2.96)	0.66	0.78 (0.22–2.76)	0.70
Age at injury, each increase of 1 year	1.09 (1.04–1.14)	0.0006	1.07 (1.03–1.11)	0.0006
BMI, each increase of 1 unit	0.97 (0.87–1.08)	0.59	0.97 (0.87–1.07)	0.50
Transport, helicopter versus Ambulance	3.36 (1.02–11.13)	0.05	2.09 (0.71–6.20)	0.18
Time POI to 1st blood drawn, each increase of 1 min	1.00 (0.99–1.01)	0.67	1.00 (0.99–1.01)	0.44
GCS, each increase of 1 scale	0.81 (0.72–0.91)	0.0003	0.86 (0.78–0.95)	0.002
Burn %TBSA	1.07 (1.04–1.11)	<0.0001	1.05 (1.03–1.07)	<0.0001
Inhalation injury, yes versus no	4.04 (1.23–13.25)	0.02	2.60 (0.94–7.21)	0.07
ADM SDC-1, each increase of 1 ng/mL	1.03 (1.01–1.05)	0.005	1.01 (1.01–1.02)	<0.0001
>34 ng/mL versus ≤ 34 ng/mL	68.60 (8.07–582.86)	0.0001	33.50 (4.40–255.03)	0.0007
ADM TFPI, each increase of 1 ng/mL	1.02 (1.00–1.04)	0.03	1.02 (1.00–1.03)	0.05
>73 ng/mL versus ≤ 73 ng/mL	5.44 (1.39–21.33)	0.02	3.76 (1.06–13.34)	0.04
Adjusted models: adjusted for age, TBSA, and *inhalation injury (SDC-1)				
ADM SDC-1				
>34 ng/mL versus ≤ 34 ng/mL	32.65 (2.67–399.78)	0.006	14.08 (1.70–116.60)	0.01
TFPI on ADM				
>73 ng/mL versus ≤ 73 ng/mL	9.59 (1.02–89.75)	0.002	2.55 (0.71–9.15)	0.15

ADM indicates admission; Syndecan-1; BMI, body mass index; GCS, Glasgow Coma Scale; POI, point of injury; TBSA, total body surface area; TFPI, tissue factor pathway inhibitor.
*Adjusted in logistic regression only.

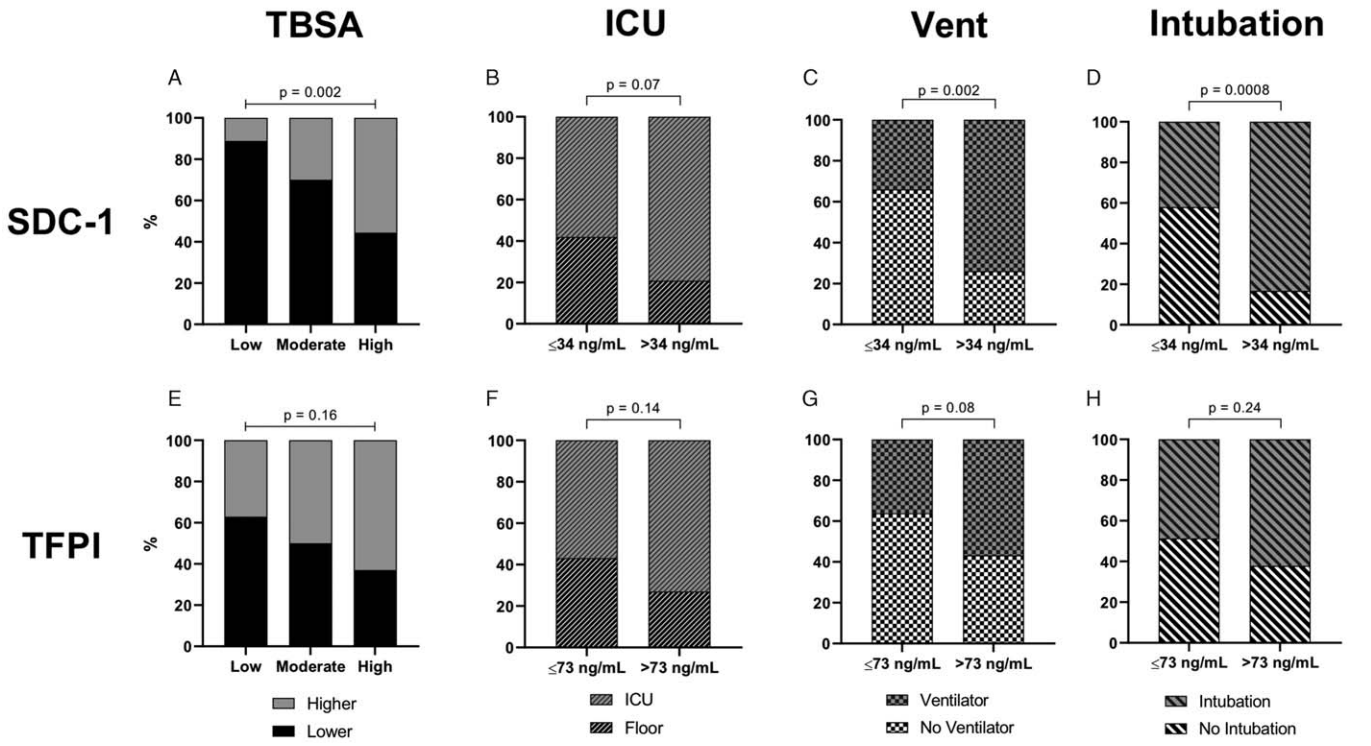

FIG. 3. **Admission SDC-1 (A–D) and TFPI (E–H) association with burn size, ICU ADM, mechanical ventilation, and intubation.** Higher and lower defined by SDC-1 and TFPI cutoffs of 34 ng/mL and 73 ng/mL respectively. ADM indicates admission; ICU, intensive care unit; SDC-1, syndecan-1; TFPI, tissue factor pathway inhibitor.

DISCUSSION

The ability to determine the degree of endotheliopathy experienced by patients after burn injury could provide clinicians with prognostic information and help guide clinical decision making. Previous work has shown that SDC-1 levels can predict patients at risk for intubation after large volume resuscitation in septic shock (4). In cardiogenic shock, SDC-1 and other indicators of endothelial damage and activation (e.g., thrombomodulin and sE-selectin) have been shown to be independently associated with the severity of post-cardiac arrest syndrome (6). Trauma researchers have proposed a SDC-1 cutoff to identify patients with endotheliopathy and have found worse outcomes in patients with levels above 40 ng/mL (1). These findings highlight the potential of using SDC-1 as a quantitative biomarker of endotheliopathy.

In this study, we demonstrate that ADM SDC-1 and TFPI levels can be used as independent predictors of 30-day in-hospital mortality in burn patients. SDC-1 performed better than TFPI in predicting mortality. SDC-1 performed as well as the revised Baux score, an established clinical measure, which suggests that circulating SDC-1 levels are directly associated with injury severity in burn patients and validates the efficacy of this biomarker. Calculation of the revised Baux score requires experience with %TBSA burn estimation and the diagnosis of inhalation injury, which is not always straightforward. Therefore, SDC-1 could provide more reliable prognostic information, especially for non-burn clinicians. An admission SDC-1 level of >34 ng/mL identified patients at risk for mortality with high sensitivity and specificity. Interestingly, this cutoff is lower than the previously established cutoff of 40 ng/mL that identified trauma patients at risk for mortality (1). The SDC-1 cutoff of 34 ng/mL is above healthy control plasma levels reported in several studies (27–30). These findings suggest that SDC-1 is effective in characterizing endotheliopathy in burn patients.

The implications of elevated TFPI in the present study are not clear. Plasma TFPI may be elevated due to loss of endothelial binding sites coincident with endothelial SDC-1 shedding, due to release from platelets, or both. The weak correlation observed between ADM SDC-1 and TFPI levels suggests that both reservoirs of TFPI contribute to plasma concentrations following burn injury. A proportion of the measured circulating TFPI is likely bound to cleaved SDC-1; however, we would expect a stronger correlation between biomarkers if TFPI levels were an indirect measure of SDC-1 shedding. Circulating TFPI may exert an inhibitory effect on systemic thrombin generation while disruption of the EGL and loss of TFPI in the area of injury could result in a local hypercoagulable environment (31). Such a phenomenon would be consistent with the acute hypocoagulable state observed in patients with burn injury in some studies (32). The present findings are consistent with animal data demonstrating increased TFPI activity in plasma from rats following experimental burn injury (23). In a cohort study of nine burn patients with TBSA >25%, Tejiram et al. found elevated TFPI levels in patients who died compared with survivors as early as 36 h after injury (33, 34). However, Kowal-Vern et al. (35) reported that TFPI levels remained within normal range on days 1 through 20 post-burn, in a study of 15 patients with an average burn size of 45% TBSA. The differing results may be due to a difference in

the timing or methods of blood collection. In the present study, samples were collected within 4 h of injury versus within 24 h. The use of collection tubes with specialized proteinase inhibitors in the present study likely increases the stability of plasma relative to citrated samples. Our proposed cutoff of 73 ng/mL is above mean healthy control plasma TFPI levels reported in the literature (36).

The link between inflammation, coagulopathy, and endothelial dysfunction has been well described. Inflammatory mediators tend to increase procoagulant, decrease anticoagulant, and inhibit fibrinolytic potential, shifting the hemostatic balance toward coagulation (37). Burn injury is characterized by an acute inflammatory response with severe injury resulting in a persistent and maladaptive pattern of systemic inflammation (38). Matsuura et al. (39) identified a cytokine network including IL-6, IL-8, MCP-1, and TNF-α that is elevated in burn patients relative to healthy controls, and significantly higher among non-survivors as early as hospital day 1. The effect of this cytokine network was investigated on endothelial cells *in-vitro* and IL-6 trans-signaling was found to increase the expression of several pro-inflammatory cytokines as well as a major fibrinolytic inhibitor (40). Furthermore, thrombin has been shown to cleave SDC family ectodomains which in turn increase endothelial cell hyperpermeability *in-vitro* and *in-vivo* (41). Syndecans play an important role in regulating inflammation by binding inflammatory molecules and regulating leukocyte adhesion at the vascular endothelium (42). Loss of this important barrier function by proteolytic cleavage of glycocalyx constituents results in increased circulating levels of SDC-1 (43, 44). It is through these interrelated mechanisms that we hypothesize circulating SDC-1 serves as a biomarker in severe burn injury. In the present study there was no relationship observed between patients with high and low SDC-1/TFPI and rTEG parameters. While not the primary focus of this study, the relationship between endothelial dysfunction, coagulation, and inflammation needs to be further elucidated.

Our results show that ADM SDC-1 and TFPI are higher in patients who die. When adjusting for possible confounders including age, burn size, and inhalation injury, our proposed SDC-1 cut-off level (>34 ng/mL) conveys a high likelihood of mortality, shorter time to death, and better fit than TFPI (>73 ng/mL). We also demonstrate an association with SDC-1 levels and burn size. These data suggest that the degree of endothelial dysfunction following burn injury is dependent on burn size, and that this relationship can be observed as early as hospital admission. A potential consequence of these findings is that early targeted treatment of endotheliopathy might improve outcomes. Interventions such as plasma-based resuscitation, which may restore the glycocalyx, could be informed by a biomarker assessment of burn injury severity (22, 30). It is important to note that no patients in the present study received blood products, including fresh frozen plasma and red blood cells, prior to blood draw.

When looking at secondary outcomes, SDC-1 performed better than TFPI at identifying patients who required emergent prehospital or on-arrival intubation, would require mechanical ventilation, or ICU admission. Given the performance of SDC-1 as an independent predictor of mortality, a predictor of interventions and critical care requirements, and its relationship with burn injury severity, SDC-1 should be the focus of further investigation of burn-induced endotheliopathy. Furthermore, infectious complications and sepsis are a significant source of morbidity and mortality in hospitalized burn patients (38). Circulating SDC-1 levels are increased in a variety of septic states and associated with poor outcomes (4, 45). In a burn-injured mouse model, investigators identified shed SDC-1 as a host factor exploited by Pseudomonas Aeruginosa, a common and devastating burn wound pathogen (18). While the current analysis focuses on admission blood samples and the acute phase of care, future studies should investigate SDC-1 as a biomarker for sepsis in burn patients during the prolonged phase of care.

Our study has several limitations. The data represents a single-institution experience with a relatively small sample size. However, we meet expectations of a power of 80% and 95% confidence intervals. Our cohort is mostly male, with a median age of 41.5 years, non-survivors were older, with higher TBSA, and more concomitant inhalation injury. While these demographics generally reflect burn injury epidemiology, they may impact the generalizability of our results. These limitations highlight the need for larger, multicenter studies. While endothelial dysfunction, inflammation, and coagulopathy contribute to shock states, organ failure, and ultimately death, this study is observational in nature and therefore describes a correlation of these outcomes with circulating biomarkers, not causation. We chose to investigate endothelial biomarkers on admission as early prognostic information is clinically relevant. However, the longitudinal course of endotheliopathy following burn injury is not well defined, and this is an area for future research.

CONCLUSION

Biomarkers of endotheliopathy, SDC-1, and TFPI are higher in burn patients who die. Reliable assessment of the degree of a patient's endothelial damage holds predictive value for clinicians and could also assist in clinical decision making, such as in the choice and timing of resuscitative or transfusion products. SDC-1 and TFPI are independent predictors of 30-day in-hospital mortality. We propose an admission SDC-1 cutoff of 34 ng/mL to predict burn patients at significant risk for mortality who are more likely to require timely critical care interventions.

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