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RPPR Final Report
as of 26-Jan-2022

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STEM Degrees: **STEM Participants:**

Major Goals: We aimed to (1) Further characterize and confirm the biphasic hypo- to hypercoagulable state shift phenomena measured in samples from previously enrolled patients and (2) Pursue endothelial dysfunction as an unexplored player in burn-induced coagulopathy.

Accomplishments: We successfully measured SDC-1 in patient samples in an attempt to identify endothelial dysfunction in patients. What we discovered is that SDC-1 is associated with a higher risk of 30-day mortality. We therefore proposed the measurement of SDC-1 on admission to identify burn patients at high risk of mortality who require timely interventions. However, further investigation with a larger sample size is warranted.

We also examined the proposed thromboelastography approaches in the sample sets. We preliminarily concluded that the choice of TEG modality may impact clinical decision-making as TEG assays may give discordant results on identical samples. rTEG was the most sensitive in its parameter-specific associations, however, no TEG assay performed better in predicting mortality. Therefore, rTEG may be the assay of choice for burn patients given its relative convenience and availability.

Training Opportunities: Throughout the course of the project, multiple surgical residents and medical students participated in data analysis and were able to compose abstracts for scientific meetings.

Results Dissemination: Findings from the study have been included in presentations in venues such as the American Burn Association annual meeting, the MHSRS, and Shock Society.

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

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Project Contribution:
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RPPR Final Report
as of 26-Jan-2022

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W911NF-17-1-0594
Biphasic Coagulopathies and Endotheliopathy in Burn Injured Patients: An Analysis of Mechanisms and Interplay
PI: Shupp, MedStar Health Research Institute

Final Technical Report
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INTRODUCTION AND PREVIOUS WORK

While a limited body of literature exists on coagulopathies in trauma patients, understanding of abnormal coagulation in burn patients remains far more limited. Some previously published research suggests that thermal injury pushes burn patients towards a hypercoagulable state via an increase in pro-thrombotic and inflammatory events while simultaneously suppressing anticoagulant and fibrinolytic pathways[1, 2]. Clinically, the incidence of thrombotic complications in burn-injured patients is lower than expected. However, there is suspicion that the minute pathophysiology associated with coagulation is not captured by current clinical methodology, frequently limited only to laboratory values such as PT, PTT, and INR, which may add to the morbidity and mortality in burn patients[2-4]. These laboratory measurements do not account for clotting factor dynamics or clot characteristics. Studies have shown alterations in antithrombin, protein C and protein S levels after burn, but controversy remains over whether burn injury induces coagulopathy[5, 6]. There is no consensus on whether burn patients with variable injury severity are at risk for hyper- or hypocoagulation. Previous prospective observational clinical studies at our institution and others, have been conducted in order to obtain a more real time and comprehensive assessment of a patient's coagulation profile, which may help clinicians better understand the pathophysiology underlying abnormal coagulation in burn patients.

The goals of the previously completed studies (prior to award W911NF-17-1-0594) were to longitudinally characterize potential mechanisms of, and outcomes resulting from, coagulopathies in burn-injured patients. Patients were enrolled with blood sample sets collected and processed in real time, and then assays carried out for clotting factor levels and function, and inflammatory markers. Additional deliverables included thromboelastometry data, and a database of clinical information abstracted and compiled from enrolled patient medical records.

The study demonstrated that contrary to some conflicting literature, burn patients experience changes in clotting status after injury. Extensive changes not identified by clinical laboratory measures (PT, INR) were seen following burn injury that may explain perturbed coagulation in these patients. Specifically, dynamic shifts appear at both the molecular and physiologic levels. Our data illustrated that burn patients exhibit hypercoagulable states and the timing and the severity of these hypercoagulable states are patient specific and may not necessarily correspond with the severity of burn injury. Using viscoelastic assays, we have shown an increase in both clot kinetics and strength which indicate rapid fibrin formation and excessive platelet activity and a potential risk for thrombosis. Endothelial dysfunction may also play a role in clotting dynamics in response to burn injury in this patient population.

PRESENT STUDIES

The focus of the current study (W911NF-17-1-0594), now complete, was to gain insight into burn-induced glycocalyx damage and its downstream effects essential to understanding shock and refining resuscitation regimens in burn patients. This work expands the field by exploring

quantification and temporality of damage to the glycocalyx following burn injury, which is critical to understanding the impact of endotheliopathy on clinical paradigms and patient outcomes. Delineating the effects of thermal injury on endotheliopathy, including the potential interplay of vascular permeability, inflammation and coagulation, will guide future therapeutic interventions to maintain vascular integrity in patients by moderating glycocalyx shedding. Further, this may help to elucidate the pathways that uncouple, resulting in the previously described development of hypercoagulable conditions in burn patients.

The studies described here were completed on the existing sample (and data) archive generated from the previously described prospective observational trial. We aimed to ***(1) Further characterize and confirm the biphasic hypo- to hypercoagulable state shift phenomena measured in samples from previously enrolled patients and (2) Pursue endothelial dysfunction as an unexplored player in burn-induced coagulopathy.***

Methods

Sample selection

Preserved citrated plasma aliquots and RNA samples generated from patient enrollment and serial blood collection from the previously described study were accessed.

ELISA

A commercially available sandwich ELISA kit for human CD138, Syndecan-1 (Diaclone, France) was used to assay for syndecan levels in previously preserved citrated plasma aliquots. 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 multi-donor plasma pool from healthy individuals, kit controls and/or in-house standards on each assay plate.

TEG

Thawed aliquots of citrated plasma were brought to room temperature and added to cups for viscoelastic assay as described in the machine manufacturer's protocols (Haemonetics). For TEG, initiators were native, RapidTEG reagent, or Kaolin. Data (and normal ranges for comparison) will include the time to clot formation (R, normal: 0-1 min), the time to achieve 2mm of clot strength (K, normal: 1-2 min), the rate of clot formation (angle, normal: 66-82 degrees), the maximum strength of the clot (MA, normal: 54-72 mm), and the degree of fibrinolysis (LY30, normal: 0-8 min). Coagulopathy identified by viscoelastic measures will be defined as two or more abnormal values out of range on a standard set of TEG values.

Statistical Analyses

Data were first examined for outliers, missing data and anomalies. Data were analyzed by t-test, ANOVA or Mann-Whitney U to assess for changes over time compared to baseline, and differences between injury severity groups. To integrate clinical variables with molecular and viscoelastic assay results, correlations were tested by Pearson's, Spearman's, or partial correlations. Analyses controlled for multiple comparisons (e.g., Bonferroni correction, permutation resampling). TEG data were analyzed to determine concordance between

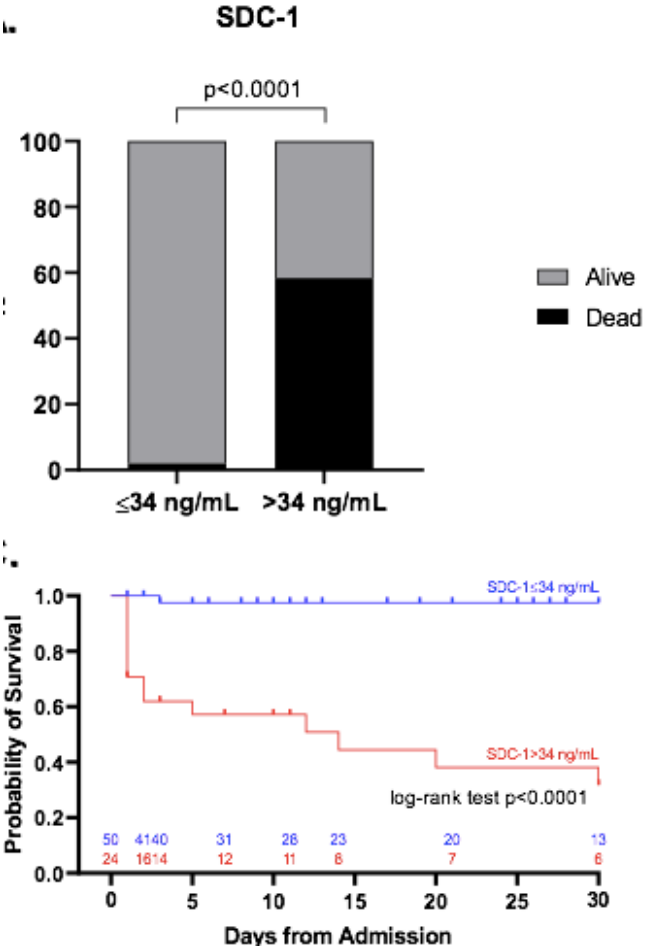
activator/assays and assess whether differences in results may be seen dependent upon assay used.

RESULTS

Most patients with samples analyzed in the present study (n=74 for syndecan, n=121 for TEG) were male with median age of 41.5 or 40 years and burn TBSA of 20.5% or 13% respective for syndecan assays and TEG.

Endothelial Dysfunction/Syndecan Quantification

The overall mortality rate was 20.3%. Admission SDC-1 was 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 (AUROC 0.92).



TEG

Overall concordance correlation coefficients for all parameters were highest between nTEG and kTEG (R-time: 0.35, α-angle: 0.55, MA: 0.82, LY30: 0.71) and lowest between nTEG and rTEG (R-time: 0.02, α-angle: 0.22, MA: 0.65, LY30: 0.63). There was poor agreement between TEG assays on R-time and α-angle and moderate to strong agreement on MA, and LY30. Interclass reliability was low for R-time and α-angle and moderate for MA and LY30. Admission MA was associated with higher mortality on all three assays (OR 0.93-0.94, p<0.05). On rTEG, admission α-angle (OR 0.93, p <0.05) and admission hyperfibrinolysis (OR 10.45, p< 0.05) was associated with higher mortality. There were no significant differences between TEG assays in predicting mortality using admission MA or LY30.

Concordance Correlation Coefficient (Rc) among nTEG, kTEG and rTEG at Admission and during 21 days

TEG parameter	nTEG & kTEG	nTEG & rTEG	kTEG & rTEG
Reaction time (R)	Rc (95% CI)	Rc (95% CI)	Rc (95% CI)
H0	0.56 (0.47-0.63)	0.001 (-0.06- 0.07)	0.01 (-0.03 - 0.04)
All	0.35 (0.33-0.37)	0.02 (0.01-0.06)	0.05 (0.02-0.08)
Rate of clot strength (α)			
H0	0.55 (0.40-0.67)	0.40 (0.24-0.54)	0.69 (0.57-0.78)
All	0.55 (0.51-0.59)	0.22 (0.18-0.25)	0.50 (0.46-0.55)
Maximum Amplitude			
H0	0.81 (0.73-0.87)	0.70 (0.60-0.79)	0.67 (0.55-0.77)
All	0.82 (0.80-0.84)	0.65 (0.62-0.69)	0.75 (0.72-0.77)
Clot lysis			
H0	0.72 (0.61-0.80)	0.92 (0.89-0.95)	0.93 (0.90-0.95)
All	0.71 (0.68-0.74)	0.63 (0.60-0.67)	0.69 (0.65-0.72)

Abbreviations: TEG, thromboelastography; nTEG, native TEG; kTEG, kaolin TEG; H0, admission time

CONCLUSIONS

SDC-1 is 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 who require timely interventions. However, further investigation with a larger sample size is warranted.

Choice of TEG modality may impact clinical decision-making as TEG assays give discordant results on identical samples. rTEG was the most sensitive in its parameter-specific associations, however, no TEG assay performed better in predicting mortality. Therefore, rTEG may be the assay of choice for burn patients given its relative convenience and availability.

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