

ELEVATED PLASMA LEVELS OF MATRIX METALLOPROTEINASE-3 AND TISSUE-INHIBITOR OF MATRIX METALLOPROTEINASES-1 ASSOCIATE WITH ORGAN DYSFUNCTION AND MORTALITY IN SEPSIS

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ABSTRACT—Background: Matrix Metalloproteinases (MMP) respond to tissue damage during sepsis. Higher plasma concentrations of MMPs and the tissue-inhibitor of matrix metalloproteinases (TIMP) have been reported in sepsis compared with healthy controls. The objective of this study was to examine if plasma levels of MMP-3, MMP-9, and TIMP-1 associate with mortality and organ dysfunction during sepsis. **Methods:** We conducted a prospective cohort study of critically ill patients with sepsis adjudicated per Sepsis-3 criteria at a tertiary academic medical center. We measured plasma concentrations of MMP-3, MMP-9, and TIMP-1 on intensive care unit admission. We phenotyped the subjects for shock, acute respiratory distress syndrome (ARDS), acute kidney injury (AKI), and mortality at 30 days. We used logistic regression to test the associations between the MMPs and TIMP-1 with shock, ARDS, AKI, and mortality. **Results:** Higher plasma TIMP-1 levels were associated with shock (odds ratio [OR] 1.51 per log increase [95% CI 1.25, 1.83]), ARDS (OR 1.24 [95% CI 1.05, 1.46]), AKI (OR 1.18 [95% CI 1.01, 1.38]), and mortality (OR 1.20 [95% CI 1.05, 1.46]). Higher plasma MMP-3 concentrations were associated with shock (OR 1.40 [95% CI 1.12, 1.75]) and mortality (OR 1.24 [95% CI 1.03, 1.48]) whereas MMP-9 levels were not associated with outcomes. Higher plasma TIMP-1 to MMP-3 ratios were associated with shock (OR 1.41 [95% CI 1.15, 1.72], $P=0.02$). **Conclusion:** Elevated plasma concentrations of TIMP-1 associate with organ dysfunction and mortality in sepsis. Higher plasma levels of MMP-3 associate with shock and mortality. Plasma MMP and TIMP-1 may warrant further investigation as emerging sepsis therapeutic biomarkers.

KEYWORDS—Acute kidney injury, acute respiratory distress syndrome, mortality, sepsis, shock

INTRODUCTION

Sepsis is a leading cause of critical illness and mortality (1). Sepsis is defined by life-threatening organ dysfunction and a dysregulated host response to an infection (2). Although inflammation and tissue damage are hallmarks of sepsis, the specific mechanisms linking inflammation, injury, and outcomes remain unclear (3). Sepsis-associated organ failures, such as acute respiratory distress syndrome (ARDS) and acute kidney injury (AKI), are associated with a substantial disease

burden including an increased risk of in-hospital death (4, 5). However, only a subset of patients with sepsis develops organ failure. The heterogeneity in host response contributes to divergent sepsis outcomes and remains a significant factor complicating the development of therapeutic interventions to improve survival (1, 6). Biomarkers for sepsis prognostication may address this problem by identifying the patients who are at high risk for adverse outcomes (3, 7–9). To further characterize the dysregulated host response in sepsis and to enhance the potential for prognostic enrichment in future clinical trials of sepsis therapeutics, we investigated the association of biomarkers from the matrix metalloproteinase (MMP) pathway with sepsis-associated organ failure, shock, and mortality.

Matrix metalloproteinases are zinc-dependent endopeptidases that primarily degrade components of the extracellular matrix (ECM) but also act on cell surface proteins that drive sepsis-associated inflammation (10). MMPs play a crucial and context-dependent role in the host response to sepsis. Damage-associated molecular patterns (DAMPs) are released from damaged and dying cells and act as a component of the innate immune response to infection. DAMPs have been demonstrated to interact with Toll-like receptors, activate the inflammasome, and potentiate the expression of cytokines and enzymes that regulate ECM homeostasis such as matrix metalloproteinases (11, 12). By degrading the ECM, MMPs can propagate the inflammatory response by facilitating neutrophil migration and recruitment into peripheral tissues

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(13). Through modifications to non-ECM proteins, the proteolytic activities of MMPs may conversely downregulate pro-inflammatory cytokine and chemokine function (14, 15). In the healthy state, the activity of plasma matrix metalloproteinases is tightly regulated (16). In an inflammatory state, tissue and circulating levels of matrix metalloproteinases are elevated by the presence of cytokines such as tumor necrosis factor- α and transforming growth factor- β (17). Prior studies have associated plasma concentrations of MMP-3, a stromelysin capable of digesting collagen, and MMP-9, a gelatinase, with sepsis-related mortality (13, 18–20). Primed neutrophils, which have the capacity to release MMPs into the circulation, are reported as key contributors to the pathophysiology of ARDS during sepsis (21). Bacterial endotoxin drives the release of cytokines which increase the expression and release of MMPs from neutrophils (22). MMP-9 has been reported as a significant factor in the progression of ARDS-specific neutrophil activation (18). Direct measurements of MMPs in the circulation have been proposed as indicators of neutrophil priming and tissue damage in sepsis (18, 21).

The activity of MMPs is counterbalanced by their naturally occurring inhibitors, tissue inhibitors of matrix metalloproteinases (TIMPs) (23). The balance of MMPs and TIMPs in the local tissue is critical in maintaining the structural integrity of the ECM during sepsis, and perturbations in ECM functioning have been implicated in respiratory diseases such as ARDS (23–25). Prior studies have demonstrated that TIMP1 performs well as a predictor of sepsis-related mortality, and higher TIMP-1 to MMP-9 ratios during the first week of sepsis have been identified in sepsis non-survivors (26). There remains a paucity of data addressing the relationship between MMPs and TIMPs with sepsis-associated organ failures in cohort studies of medical ICU patients. In a single-center prospective cohort study of critically ill sepsis patients, we sought to examine if admission plasma levels of MMP-3, MMP-9, and TIMP-1 associate with shock, organ dysfunction, and mortality. If biomarkers from the matrix metalloproteinase pathway are associated with shock, organ dysfunction, and mortality within a population of patients with sepsis, then this knowledge could have the potential to enhance our understanding of sepsis pathophysiology, facilitate prognostication and secondary prevention, and guide prognostic enrichment for future clinical trials of novel sepsis therapies.

PATIENTS AND METHODS

Study population

We conducted a prospective cohort study of subjects admitted via the emergency department and enrolled in the Molecular Epidemiology of Sepsis in the ICU cohort at the University of Pennsylvania from September 2008 through February 2015 (27). Eligible patients were admitted to the medical intensive care unit with sepsis in accordance with Sepsis-2 criteria as previously described (28). Major exclusion criteria included an alternative etiology for organ failure, a decision to pursue comfort care measures exclusively on admission, or prior enrollment into the cohort. The study was approved by the Institutional Review Board of the University of Pennsylvania with a waiver of timely informed consent. Informed consent was obtained from subjects or their healthcare proxies for all subjects in this study, and subjects were able to withdraw at any time.

Data collection

We abstracted the clinical and laboratory parameters from the electronic medical record using structured case report forms (27, 29). The case report forms were used to gather baseline demographics, chronic health information as

well as pharmacological, microbiological, and laboratory data. All chest imaging studies for the first 6 days following ICU admission were evaluated as previously described (27, 29).

Exposure and outcome classification

Sepsis was prospectively defined using Sepsis-2 criteria as adjudicated by a panel of intensivists and later retrospectively reviewed to confirm adherence to the Sepsis-3 criteria. We described shock as vasopressor use or mean arterial pressure <65 mm Hg despite 30 mL/kg fluid resuscitation within the first 24 h of intensive care unit admission. Patients were followed for 6 days to assess for the outcome of ARDS and AKI. We defined ARDS in accordance with the Berlin criteria requiring that chest radiograph and oxygenation criteria be fulfilled on the same calendar day while the patient was invasively ventilated (30). The Kidney Disease Improving Global Outcomes creatinine and renal replacement therapy criteria were applied to define AKI (31). Mortality was determined at 30 days from admission to the intensive care unit.

Plasma protein quantification

We utilized enzyme-linked immunosorbent assays (ELISA) designed for human plasma samples and tested plasma collected at emergency department (ED) presentation for patients admitted to the ICU to measure MMP-3, MMP-9, and TIMP-1 (R&D Systems, Minneapolis, Minn).

Statistical analysis

Baseline clinical characteristics were compared between sepsis survivors and non-survivors at 30 days. Categorical data were compared using Pearson chi-square test or Fisher exact test. Continuous data were compared using Student *t* or Wilcoxon rank-sum test. Plasma protein concentrations were analyzed using Wilcoxon rank-sum test. We applied multivariable logistic regression to test for associations between the plasma protein concentrations and the sepsis outcomes of shock, ARDS, AKI, and mortality. Potential confounders associated with either the exposure or outcome were removed with $P > 0.2$ and added with $P < 0.1$ to the logistic regression modeling using forward stepwise selection. Confounders such as age, sex, race, and Acute Physiology, Age, and Chronic Health Evaluation (APACHE) III score, immunocompromised state, malignancy, cirrhosis, and pulmonary source of infection were included in the regression model fitting based on prior reports of associations with outcomes in sepsis (32). When a biomarker demonstrated a significant association with the sepsis outcomes adjusting for potential confounders, we applied post-estimation marginal analysis of the final logistic regression model to determine the standardized adjusted risk ratios of the adverse outcome at several percentiles of the plasma biomarker as compared with the first percentile. Since neutrophils have been identified as a key cell type driving MMP production (33), we assessed whether admission neutrophil count, quantified as absolute neutrophil count, acted as an effect modifier for the significant associations between plasma MMP levels and outcomes. Based on a prior report of TIMP-1/MMP-9 ratio associating with higher mortality risk, we also assessed for associations between the log-transformed TIMP-1/MMP-3 and TIMP-1/MMP9 ratios and sepsis outcomes. We used the Pearson correlation coefficient to assess for the linear associations between log transformed TIMP-1 and the two matrix metalloproteinases. Plasma biomarker concentrations for all analyses were log transformed for normality and significance was assessed at two-sided $\alpha = 0.05$.

RESULTS

Clinical characteristics

We enrolled 334 patients with sepsis admitted from the ED to the intensive care unit. Compared with sepsis survivors, sepsis non-survivors were older and had a higher average APACHE III score (Table 1). Our population had a high proportion of subjects with an immunocompromised state and malignancy reflecting our center's concentration on tertiary care (Table 1). As expected, sepsis non-survivors had higher incidences of shock, ARDS, AKI, and mortality (Table 1).

Plasma protein analysis

Higher plasma levels of TIMP-1 associated with shock (odds ratio [OR] 1.51 [95% CI 1.25, 1.83], $P < 0.01$), AKI (OR 1.18

TABLE 1. Baseline characteristics of study population (n = 334)

Demographic	Survivors n = 163	Non-survivors n = 171	P value
Age at enrollment, years	59 (49–68)	63 (55–70)	<0.01*
Female sex, n (%)	71 (48.3)	76 (51.7)	0.71
Race, n (%)	—	—	0.03*
White	82 (43.2)	108 (56.8)	
Black or African American	73 (55.7)	58 (44.3)	
Asian	1 (50.0)	1 (50.0)	
Other	7 (63.6)	4 (36.4)	
APACHE III score	70 (52–94)	86 (83–102)	
Pulmonary sepsis, n (%)	73 (47.1)	82 (48.0)	0.56
Co-morbidities, n (%)	—	—	
Immunocompromised	65 (39.8)	94 (55.0)	<0.01*
Malignancy	35 (21.5)	71 (41.5)	0.03*
Cirrhosis	9 (6.3)	24 (16.7)	<0.01*
Diabetes	51 (31.3)	48 (28.1)	0.52
HIV/AIDS	1 (0.6)	6 (3.5)	0.07
CKD	34 (20.9)	31 (18.5)	0.51
Outcomes, n (%)	—	—	
ARDS	44 (28.2)	105 (62.8)	<0.01*
AKI	103 (64.0)	136 (80.5)	<0.01*
Shock	73 (44.8)	104 (61.9)	0.01*

Continuous variables are denoted as median with interquartile range. Categorical variables are denoted with count and percentage. Continuous data were compared using Wilcoxon rank-sum test. Categorical data were compared using the Chi-square test. The symbol (*) denotes that a P value is statistically significant at the $\alpha = 0.05$ level.

AKI indicates acute kidney injury; APACHE, acute physiology, age, and chronic health evaluation; ARDS indicates acute respiratory distress syndrome.

[95% CI 1.01, 1.38], $P = 0.04$), ARDS (OR 1.24 [95% CI 1.05, 1.46], $P = 0.01$), and mortality (OR 1.21 [95% CI 1.04, 1.41], $P = 0.02$) (Table 2). The adjusted risk for each of the sepsis outcomes by percentile of plasma TIMP-1 demonstrated a higher risk of adverse outcomes with higher plasma TIMP-1 percentiles (Fig. 1). The potential confounders included in each of the models fitted by stepwise regression are depicted in the Supplemental Digital Content, <http://links.lww.com/SHK/B351>. The confounders included were immunocompromised state, underlying malignancy, cirrhosis, age, sex, race, and APACHE III score. Associations of plasma concentrations of TIMP-1 with ARDS and mortality were robust to adjustment for shock: ARDS (OR 1.25 [95% CI 1.02, 1.55], $P = 0.04$), and mortality (OR 1.38 [95% CI 1.12, 1.68], $P < 0.01$) whereas the association with AKI was attenuated by adjustment (OR 1.16 [95% CI 0.96, 1.47], $P = 0.10$). Higher plasma MMP-3 concentrations were associated with shock (OR 1.40 per log increase [95% CI 1.12, 1.75]; $P = 0.002$) and mortality (OR 1.24 [95% CI

1.03, 1.48], $P = 0.02$) whereas plasma MMP-9 levels were not associated with any outcome (Table 2). Higher plasma TIMP-1/MMP-9 ratios were associated with increased risk of shock (OR 1.18 [95% CI 1.04, 1.35], $P = 0.01$), ARDS (OR 1.14 [95% CI 1.03, 1.30], $P = 0.02$), and mortality (OR 1.19 [95% CI 1.07, 1.32], $P < 0.01$), but were not associated with AKI (OR 1.07 [95% CI 0.96, 1.19], $P = 0.21$). Plasma TIMP-1/MMP-3 ratios were associated with shock (OR 1.41 [95% CI 1.15, 1.72], $P = 0.02$) and AKI (OR 1.31 [95% CI 1.06, 1.63], $P = 0.01$), but the ratios were not associated with other sepsis outcomes (Table 3). We identified a weak-to-moderate positive linear relationship between plasma TIMP-1 and MMP-3 concentrations ($\rho = 0.38$, $P < 0.01$) (34). In contrast, there was a weak-to-moderate negative linear correlation between plasma TIMP-1 and MMP-9 levels ($\rho = -0.32$, $P < 0.01$). We did not detect significant effect modification on the association between plasma MMP-3 and shock (interaction $P = 0.34$) or mortality (interaction $P = 0.40$) by admission neutrophil count.

TABLE 2. Plasma metalloproteinase concentrations associate with organ failure and mortality

Biomarker	AKI	ARDS	Shock	Mortality
	Adjusted OR (95% CI) P value	Adjusted OR (95% CI) P value	Adjusted OR (95% CI) P value	Adjusted OR (95% CI) P value
MMP-3 pg/mL	0.97 (0.76, 1.23) 0.63	1.07 (0.90, 1.30) 0.42	1.40 (1.12, 1.75) <0.01	1.24 (1.03, 1.48) 0.02
MMP-9 pg/mL	0.92 (0.76, 1.13) 0.46	0.96 (0.81, 1.12) 0.55	0.91 (0.76, 1.09) 0.29	0.87 (0.74, 1.01) 0.07
TIMP-1 pg/mL	1.18 (1.01, 1.38) 0.04	1.24 (1.05, 1.46) 0.01	1.51 (1.25, 1.83) <0.01	1.21 (1.04, 1.41) 0.02

Logistic regression modeling was used to assess for associations between log-transformed biomarker levels and sepsis outcomes adjusting for potential confounders based on stepwise regression.

AKI indicates acute kidney injury; ARDS, acute respiratory distress syndrome; CI, confidence interval; MMP-3, matrix metalloproteinase-3; MMP-9, matrix metalloproteinase-9; OR, odds ratio; TIMP-1, tissue metalloproteinase inhibitor 1.

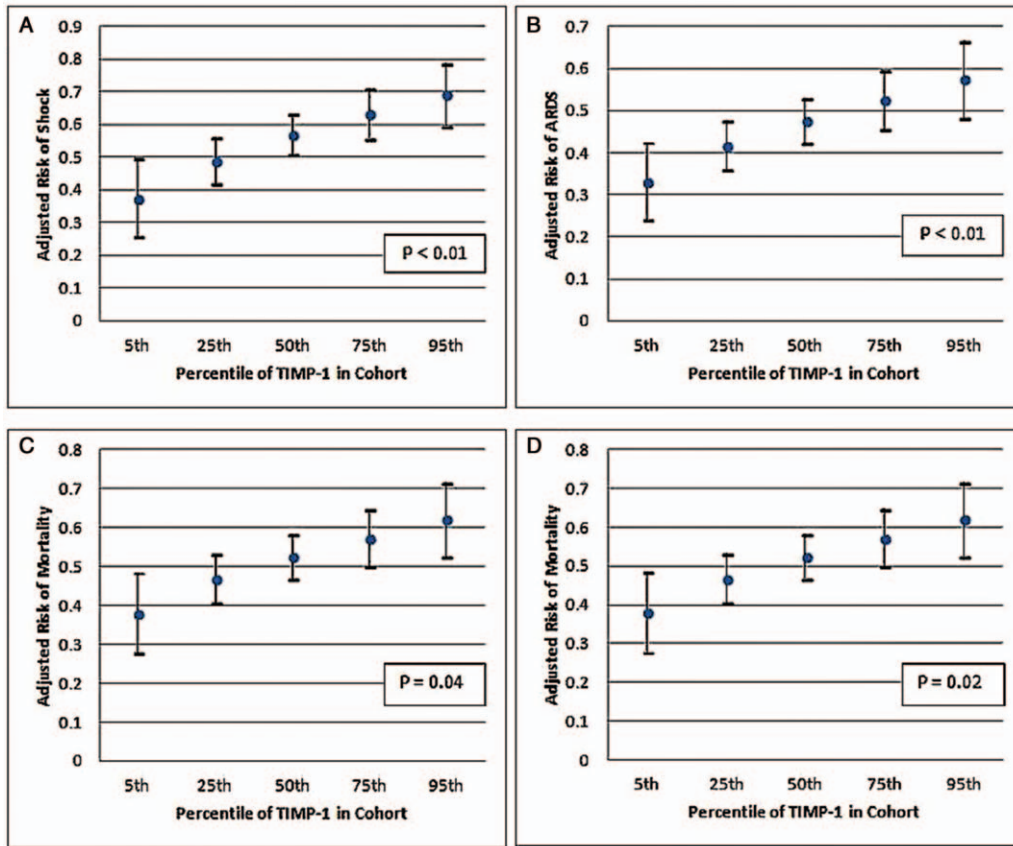


FIG. 1. Adjusted risk for sepsis outcomes increases with higher plasma TIMP-1 concentrations. Higher plasma TIMP-1 concentrations were associated with higher adjusted risks of shock (A), ARDS (B), AKI (C), and mortality (D) using postestimation marginal analysis. The 95% confidence intervals for each risk estimate are denoted by the vertical error bars. The P values are for the adjusted association between plasma TIMP-1 concentrations and each of the sepsis outcomes. AKI indicates acute kidney injury; ARDS, acute respiratory distress syndrome; TIMP-1, tissue-inhibitor of matrix metalloproteinases.

DISCUSSION

As the field of critical care medicine moves toward a pathobiology-based understanding of sepsis heterogeneity, plasma biomarkers hold promise for the identification of sepsis subgroups at high risk for adverse outcomes, and potentially for specific pathway dysregulation (3, 6). Consequently, sepsis biomarkers have the potential to improve both clinical prognostication and prognostic enrichment for clinical trials (6, 35, 36). Our study examined the role of biomarkers from the matrix metalloproteinase pathway for associations with sepsis-

associated organ failure, shock, and mortality. We demonstrate that early plasma concentrations of TIMP-1 and MMP-3 associate with sepsis outcomes including shock, ARDS, AKI, and mortality. We provide evidence of TIMP-1 as a potential prognostic biomarker for shock, ARDS, AKI, and mortality and MMP-3 as a biomarker for shock and mortality. We also determined that higher plasma TIMP-1/MMP-9 ratios are present in patients that develop shock, ARDS, and mortality as compared with sepsis patients who do not experience these complications.

TABLE 3. Plasma levels of the tissue inhibitor of matrix metalloproteinase-1 to matrix metalloproteinases ratios associate with sepsis outcomes

Biomarker	AKI Adjusted OR (95% CI) P value	ARDS Adjusted OR (95% CI) P value	Shock Adjusted OR (95% CI) P value	Mortality Adjusted OR (95% CI) P value
TIMP-1 to MMP-3	1.31 (1.06, 1.63) 0.01	1.16 (0.96, 1.40) 0.11	1.41 (1.15, 1.72) <0.01	1.08 (0.90, 1.30) 0.38
TIMP-1 to MMP-9	1.09 (0.99, 1.24) 0.15	1.14 (1.03, 1.30) 0.02	1.18 (1.04, 1.35) 0.01	1.19 (1.07, 1.32) <0.01

Logistic regression modeling was used to assess for associations between log-transformed biomarker levels and sepsis outcomes adjusting for potential confounders based on stepwise regression.

AKI indicates acute kidney injury; ARDS, acute respiratory distress syndrome; CI, confidence interval; MMP-3, matrix metalloproteinase-3; MMP-9, matrix metalloproteinase-9; OR, odds ratio; TIMP-1, tissue metalloproteinase inhibitor 1.

Prior studies conducted in patients with sepsis described the potential role of biomarkers from the matrix metalloproteinase pathways in predicting sepsis outcomes (37). Higher plasma MMP-3 levels have been described in patients with sepsis as compared with patients with trauma and stroke (38). In a study comparing patients with severe sepsis to healthy controls, an approximately 3-fold increase in plasma MMP-3, MMP-7, and MMP-8, and MMP-9 levels was detected (14). Elevated plasma levels of MMP-3 have been associated with endothelial injury and impaired oxygenation in ARDS (10). Plasma MMP-3 has also been reported to distinguish systemic inflammatory response syndrome and sepsis in combination with other factors such as the soluble receptor for advanced glycation end products, granulocyte-macrophage colony stimulating factor, and interleukin-1 β (26, 39, 40). In a prospective study of cardiac ICU patients (23), TIMP-1 levels associated with increased 30-day mortality risk and were found to be a significant prognostic biomarker for sepsis-associated organ failure following major abdominal surgery (24). Plasma TIMP-1 concentration was also described as associating with AKI and ARDS risk (10, 18). Our study extends the prior knowledge about the associations between plasma MMP-3, MMP-9, and TIMP-1 and sepsis complications by examining the odds of sepsis-associated organ failure, shock, and mortality in a prospective cohort of undifferentiated, critically-ill sepsis patients admitted to the medical ICU.

Our findings strengthen the available evidence describing associations between plasma levels of MMP-3 and TIMP-1 with sepsis complications (13, 18, 19, 23, 24, 26). As MMP-3 has been implicated in endotoxin-induced acute inflammation in an animal model (41), our findings intimate that MMP-3 may also play a crucial role in the inflammatory response to sepsis in humans. To our knowledge, we are the first to report on novel associations between the plasma TIMP-1 concentration and early shock, the plasma TIMP-1 concentration and ARDS in an adult sepsis population, the TIMP1 to MMP-3 ratio in plasma and early shock, and the TIMP-1 to MMP-9 ratio in plasma and sepsis-associated ARDS (26). We also describe the association of plasma TIMP-1 with AKI in an undifferentiated sepsis population (24).

Our findings underscore the strong association between plasma concentrations of TIMP-1 and sepsis complications including shock, organ failure, and mortality. While these associations may seem to suggest a pathogenic role for TIMP-1 in sepsis, experimental evidence in mice has implicated TIMP-1 in the attenuation of the inflammatory response through downstream effects on MMP activity and receptor-mediated signaling (42). In the inflammatory state, MMP activity contributes to tissue remodeling and pro-inflammatory signaling, which TIMPs counterbalance through MMP inhibition. Accordingly, in a mouse model, administration of recombinant TIMP-1 has been demonstrated to alleviate inflammatory hypersensitivity when delivered to the site of the injury (42). Since TIMP-1 expression and activity are upregulated by increased MMP activity (42), we hypothesize that plasma concentrations of TIMP-1 may act as a marker of dysregulated MMP response and inflammatory hypersensitivity. If plasma levels of TIMP-1 are determined to be modifiable

by therapeutic interventions targeting MMP activity, then TIMP-1 may be a crucial response indicator variable to assess for the efficacy of the inhibition of aberrant MMP activity in sepsis.

Matrix metalloproteinases inhibitors (MMPIs) have been proposed for applications to treat vascular disease and endothelial dysfunction in ARDS. MMPIs have been applied as therapy for acute ischemic events by rescuing tissue perfusion in the penumbra zones (43). Chemically modified tetracycline derivatives have been demonstrated to provide MMP inhibition and a reduction in the severity of ventilator-induced lung injury in rats (44). Doxycycline has also been reported as a beneficial MMP inhibitor in the context of ARDS (44, 45). Future research is warranted to investigate MMPIs as novel treatments for sepsis and TIMP-1 as a potential response indicator variable. As MMPIs are investigated as novel treatments for sepsis or sepsis-associated ARDS, our work suggests that plasma concentrations of TIMP-1 may serve as a theragnostic marker for clinical trials. Other potential therapeutic strategies targeting this pathway which warrant further investigation include monoclonal antibodies, cytokine antagonists, and extracorporeal cytokine adsorption (46).

Our study has important limitations. First, the results were derived from a single-center, tertiary care center that may limit the generalizability of the results. We carefully phenotyped subjects for ARDS and AKI in the first 6 days following sepsis admission, yet it is plausible that a subset of patients developed ARDS, AKI, or shock later during their hospitalization. However, it is worth noting that prior studies have indicated that the majority of incident ARDS cases occur within the first 2 days of admission (47). In this study population, we observed that 76.1% of the subjects developed ARDS within the first 2 days of admission. We also recognize that our cohort has a relatively high mortality at 48%, which may influence external validity. However, we note that this finding was likely influenced by the severity of illness of the study population. In addition, the observed mortality rate is consistent with the predicted in-hospital mortality rate between 45% and 55% that would be anticipated based on the study population's APACHE III scores as depicted in Table 1 per the APACHE III predictive Eq. (48). Circulating plasma proteins may not be reliable reporters for tissue activity, and the precise relationships between tissue resident MMPs and plasma MMPs in shaping sepsis outcomes are both unknown and difficult to study in humans with sepsis (49, 50). Yet, plasma markers have high potential for translation to clinical care. As plasma concentrations of TIMP-1 may reflect local tissue activity that inhibits multiple matrix metalloproteinases simultaneously, TIMP-1 may be a robust and reliable reporter of MMP dysregulation in sepsis. We also note that our plasma biomarkers were obtained at a single time-point, and prior studies have suggested that levels of the MMP biomarkers are dynamic in the setting of critical illness (18). Our study was not designed to detect relationships between MMP pathway markers and adverse outcomes that unfold over the course of critical illness. Nonetheless, the early time point analyzed in this study may be most informative for clinical care and prognostic enrichment into clinical trial to test preventative therapies. While we have used research quality ELISAs to

quantify the biomarker concentrations in this study, we recognize that large-scale bedside application of these biomarkers for clinical care will require rapid, widely available, and validated testing with regulatory approval. Molecular point-of-care testing techniques that aim to detect a combination of biomarkers rapidly using magnetic beads and an electrical detection system or multiplex ELISAs are currently in development (51), and we propose that TIMP-1 and MMP-3 may warrant further investigation for inclusion on such panels for sepsis. We also acknowledge that we measured a limited number of representative MMP pathway biomarkers based on prior published reports (10, 24, 26). As a result, there may be other members of the MMP pathway that have associations with sepsis outcomes that were not identified in the current study. We also report that the association between plasma concentrations of TIMP-1 and AKI is not robust to adjustment for shock, but this finding may be due to shock being in the causal pathway. Finally, as our study is designed as an observational cohort study, the purpose is to test associations, rather than causality, and accordingly our mechanistic inferences are limited as the assessment of causation would require an experimental design.

CONCLUSION

Sepsis remains a leading cause of morbidity and mortality for patients experiencing critical illness. Biomarkers derived from the pathophysiology of sepsis and that reliably associated with sepsis outcomes may expedite the identification of patients at high risk for adverse outcomes, so that these individuals may be targeted for preventative therapy (3, 52). We report statistically significant associations between plasma TIMP-1 and MMP-3 with sepsis complications. Further research is warranted to ascertain if these associations may be applied to facilitate sepsis subgroup identification for clinical trials (9). Given the emerging investigation of MMPIs as novel therapies for ARDS, this work suggests that plasma concentrations of TIMP-1 may serve as an appropriate theragnostic marker for future clinical trials. As the field of critical care medicine approaches personalized therapy for sepsis (53–55), prognostic enrichment that leverages the associations between plasma markers from well-established sepsis pathways and clinical outcomes may have therapeutic relevance and benefit future clinical trials of sepsis therapies.

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