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Plasma Levels of Proresolving and Prophlogistic Lipid Mediators: Association With Severity of Respiratory Failure and Mortality in Acute Respiratory Distress Syndrome

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Objectives: Acute respiratory distress syndrome is characterized by an overly exuberant inflammatory state in the lung. Specialized proresolving mediators are endogenous agonists for the resolution of lung inflammation and injury in health, yet their association with acute respiratory distress syndrome severity and outcomes remains to be defined. In the current study, we investigate associations between plasma levels of specialized proresolving mediators and acute respiratory distress syndrome severity and mortality.

Design: Translational pilot study nested within a large prospective cohort of patients with risk factors for acute respiratory distress syndrome.

Setting: ICU from a large medical center.

Patients: Twenty-six Caucasian patients with acute respiratory distress syndrome and available plasma from early in critical illness.

Interventions: None.

Measurements and Main Results: Here, in samples from 26 acute respiratory distress syndrome patients, we examined plasma levels of select specialized proresolving mediators that promote lung injury resolution in preclinical systems, namely lipoxin A_4 and maresin 1, and select prophlogistic lipid mediators linked to acute respiratory distress syndrome pathogenesis, namely cysteinyl leukotrienes and thromboxane B_2. These mediators were detected by sensitive enzyme-linked immunosorbent assay: lipoxin A_4 (assay range) (8.2–5,000 pg/mL), maresin 1 (7.8–1,000 pg/mL), cysteinyl leukotrienes (7.8–1,000 pg/mL), and thromboxane B_2 (15.6–2,000 pg/mL). Lower plasma levels of specialized proresolving mediators were associated with increased duration of ventilatory support and ICU length of stay. Even in this small sample size, trends were evident for increased cysteinyl leukotrienes to specialized proresolving mediator ratios (cysteinyl leukotrienes/maresin 1 and cysteinyl leukotrienes/lipoxin A_4) in acute respiratory distress syndrome nonsurvivors.

Conclusions: Lower specialized proresolving mediator levels in acute respiratory distress syndrome patients may disrupt timely resolution of lung inflammation and/or injury and contribute to clinical severity and mortality.

Key Words: acute respiratory distress syndrome; acute respiratory distress syndrome mortality; acute respiratory distress syndrome severity; inflammation resolution; specialized proresolving lipid mediators

A dysregulated and overwhelming lung inflammatory response underlies the pathogenesis of the acute respiratory distress syndrome (ARDS) (1). Bioactive lipid mediators play an important role in initiating the inflammatory response and promoting its resolution. Prophlogistic lipid mediators activate circulating leukocytes and platelets and increase endothelial permeability for neutrophil recruitment to the lung, which can result in epithelial barrier disruption and organ damage (2). Specialized proresolving mediators (SPMs) are a family of enzymatically-derived endogenous bioactive lipids that counter-regulate inflammatory responses and signal the resolution of inflammation to promote a return to homeostasis (3). Therefore, an imbalance between prophlogistic lipid mediators and SPMs may contribute to the excess lung inflammation seen in most patients with ARDS, leading to increased disease severity and worse clinical outcomes. In the current pilot study, in plasma samples from ARDS patients, we examined levels of prophlogistic lipid mediators cysteinyl leukotrienes (leukotriene C_4, leukotriene D_4, leukotriene E_4, collectively termed as Cys-LTs) and thromboxane A_2 (by measurement of its stable metabolite thromboxane B_2) that are linked to ARDS pathobiology (4, 5) and select SPMs that promote

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the resolution of lung injury in preclinical models lipoxin A_4 (LXA_4) and maresin 1 (MaR1) (6, 7), and determined if associations with ARDS mortality and severity were present.

MATERIALS AND METHODS

Biospecimens included in the current study were obtained from the Molecular Epidemiology ARDS parent study of which study design has been described in depth previously (8) (**Supplemental Methods**, Supplemental Digital Content 1 http://links.lww.com/CCX/A379). The study was performed in line with the principles of the Declaration of Helsinki. The Human Subjects Committees of the Massachusetts General Hospital and the Harvard School of Public Health approved the study and informed written consent was obtained from subjects or their surrogates. Plasma samples were collected within 48 hours of ICU admission at the Massachusetts General Hospital. For the present study, 26 ARDS patients were randomly selected from 1,127 participants with complete 28-day mortality data. Lipid mediators in plasma aliquots (0.5 mL) were extracted as previously described (5) (Supplemental Methods, Supplemental Digital Content 1, http://links.lww.com/CCX/A379). Prophlogistic and SPMs were measured by sensitive enzyme-linked immunosorbent assay in tandem following manufacturer's instructions. For comparisons between groups, Mann-Whitney *U* test, Fisher exact test, and Kruskal-Wallis nonparametric analysis of variance with post hoc Dunn multiple comparison test were used. Correlation between the variables was calculated using the Spearman test. To control for multiple testing, the false discovery rate (FDR) was used and *q* values of less than 0.05 were designated as statistical significance. All analyses were performed with GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA) and Statistical Analysis System software (v.9.4; SAS Institute, Cary, NC).

RESULTS

Demographic characteristics of the ARDS study population are described in **Table 1**. Of note, the 28-day mortality rate was 35%. Survivors and nonsurvivors exhibited no significant differences in

TABLE 1. Demographics, Clinical, and Laboratory Characteristics of Study Population

Characteristics	All Patients (*n* = 26)	ARDS Survivors (*n* = 17)	ARDS Nonsurvivors (*n* = 9)	*p*[a]
Age, median (IQR)	57 (49–61)	58 (46.2–69.5)	57 (49–59)	0.53
Male, *n* (%)	12 (46.1)	7 (41.2)	5 (55.5)	0.68
White, *n* (%)	25 (96.1)	17 (100)	8 (88.9)	0.35
ARDS risk factors, *n* (%)				
Sepsis	26 (100)	17 (100)	9 (100)	1
Pneumonia	22 (84.6)	15 (88.2)	7 (77.8)	0.60
Multiple transfusion	1 (3.8)	0 (0)	1 (11.1)	0.35
Aspiration	3 (11.5)	3 (17.6)	0 (0)	0.53
Bacteremia	8 (30.8)	4 (23.5)	4 (44.4)	0.38
Vasopressors within 24 hr admission	26 (100)	17 (100)	9 (100)	1
Diabetes, *n* (%)	7 (28)	5 (31.25)	2 (22.2)	1
Solid tumor, *n* (%)	1 (4)	1 (6.25)	0 (0)	1
Liver cirrhosis/failure, *n* (%)	3 (13)	0 (0)	3 (33.3)	0.04
History of alcohol abuse, *n* (%)	5 (19.2)	2 (11.8)	3 (33.3)	0.75
History of steroid use, *n* (%)	1 (3.84)	1 (5.9)	0 (0)	1
Acute Physiology and Chronic Health Evaluation III, median (IQR)	91 (64–102)	84 (54.5–96)	102 (82.5–117)	0.02
WBC ($\times 10^3$/μL), median (IQR)	11.15 (6.47–14.10)	10.7 (5.95–13.6)	18.2 (14.8–26.6)	0.30
Platelet ($\times 10^3$/μL), median (IQR)	138.5 (94–226.3)	165 (125.5–316.5)	96 (73.5–178)	0.07
Length of ventilatory support, d, median (IQR)	11 (5.5–17)	11 (5.25–22.25)	10 (5–13.5)	0.73
ICU length of stay, d, median (IQR)	12.5 (7.75–22.25)	13 (6.5–22)	12 (9–22.5)	0.78
Pao_2/Fio_2 (mm Hg) at ICU admission, median (IQR)	143.2 (110.9–207.3)	148 (112.1–210.5)	1,130 (70.5–200.5)	0.59
Positive end-expiratory pressure (mm Hg) at admission, median (IQR)	10 (7.37–12.5)	10 (8–14.5)	8 (6–10)	0.12
Lung injury score, median (IQR)	2.5 (1.5–3)	2.5 (1.75–3)	2 (1.25–2.5)	0.42

ARDS = acute respiratory distress syndrome, IQR = interquartile range.

[a]*p* values for ARDS survivors vs nonsurvivors comparisons.

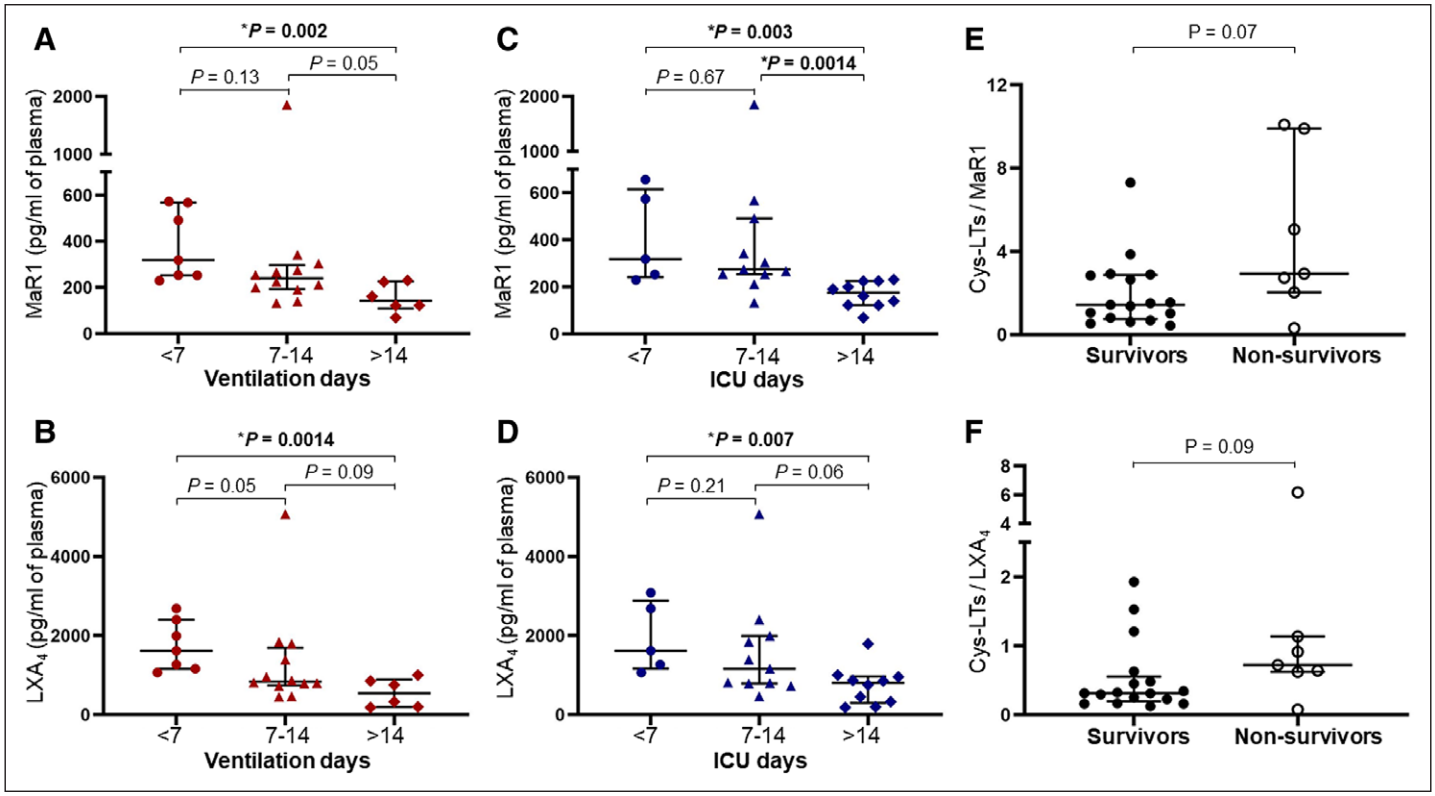

Figure 1. Lipid mediators and acute respiratory distress syndrome (ARDS) severity and mortality. Relationship between plasma levels of specialized proresolving mediators, maresin 1 (MaR1) and lipoxin A_4 (LXA_4) and the number of assisted ventilation days (**A** and **B**) and length of ICU stay (**C** and **D**). Ratios of cysteinyl leukotrienes (Cys-LTs)/MaR1 (**E**) and Cys-LTs/LXA_4 (**F**) for survivors and nonsurvivors patients with ARDS. *False discovery rate *q* value of less than 0.05.

these demographic characteristics, except for an Acute Physiology and Chronic Health Evaluation (APACHE) III score and higher rates of cirrhosis/liver failure in nonsurvivors. To investigate the possibility of selection bias for the 26 ARDS subjects for analysis here from a total of 1,127 in the molecular epidemiology of ARDS cohort, 24 demographic variables and comorbidities were used for comparative analyses (Supplemental Methods, Supplemental Digital Content 1, http://links.lww.com/CCX/A379). APACHE III score and early vasopressor use were the only variables that showed significant differences between the selected and nonselected ARDS patients with higher mean values for the selected patients (85.3 vs 75.0 APACHE III scores; $p = 0.03$ and 100% vs 71% vasopressor use; $p = 0.002$) (**Supplemental Table 1**, Supplemental Digital Content 1, http://links.lww.com/CCX/A379).

Lipid mediators were detectable at concentrations within their bioactive range. A significant negative correlation was observed between plasma levels of MaR1 and LXA_4 and duration of mechanical ventilation ($p = 0.002$; $r_s = -0.59$ and $p = 0.001$; $r_s = -0.63$, FDR $q < 0.05$) and between these SPMs and the ICU length of stay ($p = 0.0004$; $r_s = -0.64$ and $p = 0.002$; $r_s = -0.58$, FDR $q < 0.05$) (**Supplemental Table 2**, Supplemental Digital Content 1, http://links.lww.com/CCX/A379). In addition, patients with fewer than 7 ventilator days showed significantly higher MaR1 and LXA_4 values than those patients who have been mechanically ventilated for more than 14 days ($p = 0.002$ and $p = 0.0014$, FDR $q < 0.05$) (**Fig. 1**, ***A*** and ***B***) (**Supplemental Table 3**, Supplemental Digital Content 1, http://links.lww.com/CCX/A379). MaR1 and LXA_4 concentrations were also significantly increased in patients that stay in ICU for less than 7 days when compared with those with a length of stay larger than 14 days ($p = 0.003$ and $p = 0.007$, FDR $q < 0.05$) (**Fig. 1**, ***C*** and ***D***) (**Supplemental Table 4**, Supplemental Digital Content 1, http://links.lww.com/CCX/A379). Furthermore, patients that stayed in ICU for 7–14 days also showed significantly higher plasma MaR1 levels when compared with patients with longer ICU stays ($p = 0.0014$, FDR $q < 0.05$) (Fig. 1*C*). Compared with ARDS survivors, ARDS nonsurvivors showed an association with increased plasma levels of Cys-LTs ($p = 0.07$, FDR $q > 0.05$) and increased Cys-LTs/MaR1 and Cys-LTs/LXA_4 ratios ($p = 0.07$ and $p = 0.09$, FDR $q > 0.05$) (**Fig. 1**, ***E*** and ***F***) (**Supplemental Table 5**, Supplemental Digital Content 1, http://links.lww.com/CCX/A379).

DISCUSSION

While preclinical and clinical studies support the contribution of bioactive lipid mediators in the initiation and resolution of acute lung injury (ALI) (4–7, 9), the lipid mediator profiles in plasma from patients with ARDS, and their association with disease severity and outcomes is of interest to determine their potential utility as biomarkers and for subphenotyping patients.

Here, we focused on resolution mediators and found significant inverse associations between plasma levels of the SPMs LXA_4 and MaR1 and two important clinical ARDS outcome measures, namely duration of ventilatory support and length of stay in the ICU. Also notable were trends for increased plasma Cys-LTs levels and elevated ratios of Cys-LTs/MaR1 and Cys-LTs/LXA_4 in ARDS nonsurvivors. Disruption of proresolving signaling mechanisms,

especially in a manner that skews to prophlogistic signaling, seems linked to poor clinical outcomes in ARDS. Counter-regulation of lung inflammation, no matter the cause, requires both anti-inflammation and proresolution mechanisms; properties that both LXA_4 and MaR1 transduce via cognate receptors at pico-nanomolar concentrations (3). Preclinical findings in murine models of ALI suggest that decreased SPMs delay lung inflammation resolution and tissue repair, increasing severity and duration of ALI (6, 7).

This study evaluated relationships between SPM plasma levels and mortality and severity of respiratory failure in ARDS in a well-curated cohort of patients. Previous work by Maile et al (10) explored associations of the plasma lipidome with mortality in ARDS, showing decreased concentrations of polyunsaturated fatty acids (substrates for lipid mediators studied here) in ARDS nonsurvivors, supporting the notion that decreased production of proresolving mediators may disrupt engagement of endogenous resolution circuits and predispose to poor outcomes in ARDS.

ARDS represents the most severe form of pulmonary coronavirus disease 2019 (COVID-19) illness. Previous work has shown that plasma levels of SPMs are lower in patients with asthma, diabetes mellitus, hypertension and coronary heart disease, and tend to fall with age (11–13). Interestingly, COVID-19 severity is increased in those with these underlying conditions and older patients (14, 15); raising the possible association between severe COVID-19 and preexisting deficiency in SPMs. Whether SPM levels could serve as blood biomarkers for COVID-19 severity or to identify patients who would benefit from SPM supplementation is speculative, but would be of interest in future translational research.

While this pilot study identified several interesting findings in support of the concept that defective resolution mechanisms underlie ARDS pathogenesis, there are several limitations to consider. The small number of subject biospecimens analyzed limited the study's power to find significant associations (particularly between plasma lipid mediator levels and ARDS mortality). In addition, patients' comorbidities may influence circulating levels of lipid mediators. The small sample size limited our ability to conduct adjusted analyses to account for the effects of potential confounders. Furthermore, in this study, only one time point was considered in the assessment of plasma lipid mediator levels. Change in circulating lipid mediator levels over time and their relationships to ARDS outcomes remain to be determined. Further studies of a broader array of lipid mediators and with a larger sample size and multiple time points would be of interest to more fully characterize the relationships between intermediary metabolism of bioactive lipid mediators and outcome measures in ARDS.

CONCLUSIONS

In conclusion, plasma levels of bioactive lipid mediators selected based on preclinical mechanistic studies were determined and found to associate with ARDS severity and mortality. This translational pilot study is limited by sample size; however, these findings provide proof of concept for ARDS pathophysiology as a clinical condition of defective resolution and suggest that SPMs and other bioactive lipid mediators may serve as rational biomarkers for subphenotyping ARDS.

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