Alveolar MMP28 is Associated With Clinical Outcomes and Measures of Lung Injury in Acute Respiratory Distress Syndrome

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Keywords: acute respiratory distress syndrome, alveolar macrophages, matrix metalloproteinases

Posted Date: January 8th, 2020

DOI: https://doi.org/10.21203/rs.2.20294/v1

Version of Record: A version of this preprint was published at Critical Care on April 8th, 2020. See the published version at https://doi.org/10.1186/s13054-020-02847-0.

Abstract

Background

Excessive inflammation leading to increased alveolar-capillary barrier permeability remains the pathogenic model for acute respiratory distress syndrome (ARDS). Alveolar macrophage (AM) polarization has been shown to modify the activity of various matrix metalloproteinases (MMPs) that have downstream effects on key ARDS cytokines/chemokines, however the relationship between AMs, MMP28 (the newest member of the MMP family), and ARDS clinical outcomes is unknown.

Methods

We analyzed bronchoalveolar lavage fluid (BALF) and peripheral blood from subjects previously enrolled in a phase-II trial of omega-3 fatty acids for the treatment of ARDS (n = 76). In a subset of these patients (n = 25), we tested for assocations between AM- and peripheral blood monocyte (PBM)-specific MMP28 gene expression and clincal outcomes [ventilator-free days (VFDs), P a O 2 /F i O 2 ratio (P/F ratio), and sequential organ failure assessment score (SOFA)]. We tested for assocations between soluble BALF or plasma MMP28 concentrations and ARDS clinical outcomes and inflammatory mediator concentrations in the entire cohort.

Results

Increased AM MMP28 gene expression was significantly associated with worse VFDs and P/F ratio ($p < 0.05$). Higher BALF MMP28 concentrations were associated with worse P/F, but not VFDs. Increased BALF MMP28 concentrations were associated with increased % neutrophils as well as BALF total protein, IL-6, IL-17A, and MCP-1 concentrations (all $p < 0.05$). Plasma MMP28 concentrations were not associated with any clinical outcome. Increased PBM MMP28 gene expression was associated with worse P/F ratio but not VFDs.

Conclusions

Higher AM MMP28 gene expression and BALF MMP28 concentrations are associated with poor clinical outcomes and with increased alveolar inflammatory mediators in patients with ARDS.

Introduction

Acute respiratory distress syndrome (ARDS) is a clinical syndrome characterized by the acute onset of significant hypoxemia associated with bilateral pulmonary opacities on imaging that are not fully explained by cardiac dysfunction [1]. It has a major impact on global health, representing approximately 10% of total critical care admissions and 23% of all patients supported on mechanical ventilation worldwide [2]. Alveolar macrophages (AMs) are the most abundant immune cell in the homeostatic human lung [3], and recently proposed models of ARDS pathogenesis have described a key role for AM polarization in coordinating the inflammatory and reparative processes that occur in the alveolar space

after lung injury [4]. “M1-like” macrophages are induced by T helper 1 (T_H1) cytokines such as IFN-γ and are characterized by producing high concentrations of pro-inflammatory factors such as IL-1β and TNF-α. “M2-like” macrophages are induced by T helper 2 (T_H2) cytokines such as IL-4 and are characterized by producing pro-resolving mediators such as lipoxins and resolvins.

Human matrix metalloproteinases (MMPs) compose a family of 23 structurally related proteinases that alter extracellular matrix protein structure and function [5]. MMPs can significantly modify the activity and bioavailablity of important cytokines and chemokines involved in the pathogenesis of ARDS. Indeed, BALF MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9 have all been associated with the presence or severity of ARDS [6–8]. The relationship between these MMPs and ARDS may be mediated by neutrophil chemotaxis [9]. Macrophages express a unique repertoire of MMPs based on exposure to select stimuli and their polarized states such as “M1-like” or “M2-like” [10]. Hence, macrophage polarization can result in expression of MMPs that may have downstream effects on extracellular proteins, including matrix proteins, chemokines, and chemokine gradients. MMPs may also alter the activation state of the macrophage, although the MMP substrates modulating these effects remain unknown [5, 11–14].

MMP28 is the newest member of the MMP family and has been shown to be upregulated in inflammatory conditions such as idiopathic pulmonary fibrosis [15, 16] and acute myocardial infarction [17, 18]. MMP28 is expressed by both “M1-like” and “M2-like” stimulated macrophages, and we have previously shown that the absence of MMP28 is associated with a skewing of macrophage polarization toward a pro-inflammatory cell with impaired “M2-like” polarization [12, 16]. Associated with these alterations in macrophage polarization is modestly faster bacterial clearance in a murine model of Pseudomonas aeruginosa pneumonia, impaired wound healing, and protection from cigarette-smoke induced emphysema in $Mmp28^{-/-}$ mice [12, 17, 19, 20]. Despite the role MMP28 plays in murine models of lung injury, little is known about its role in human macrophages during ARDS.

AM polarization plays a central role in orchestrating alveolar inflammation and repair in ARDS [4, 21], and we have previously shown that AM transcriptional programs are associated with ARDS clinical outcomes such as ventilator-free days (VFDs) [22]. Our primary hypothesis was that AM MMP28 gene expression is associated with VFDs. Secondarily, we hypothesized that AM MMP28 gene expression and alveolar MMP28 concentrations are associated with oxygenation, overall organ dysfunction, and concentrations of alveolar cytokines and chemokines involved in ARDS pathogenesis.

Methods

Study Population

We used samples (n = 76) previously collected from subjects who were enrolled at five North American medical centers for a phase-II placebo-controlled trial of omega-3 fatty acids for the treatment of ARDS [23]. Full inclusion and exclusion criteria have been previously described [22, 23]. ARDS was adjudicated by the trial study team. Enrollment into the trial occurred within 48 hours of ARDS onset. Plasma and

bronchoalveolar lavage fluid (BALF) were collected at study entry (Day 1), day 4 ± 1 (Day 4), and day 8 ± 1 (Day 8) after ARDS onset if a subject remained alive and mechanically ventilated. On Day 1, samples were obtained from subjects prior to receiving study drug. Patients from both the placebo (n = 39) and treatment (n = 37) arms of the trial were included in our study. All studies were approved by the Human Subjects Division at the University of Washington.

Alveolar Macrophage and Peripheral Blood Monocyte Isolation

Negative selection for AMs and peripheral blood monocytes (PBMs) was performed as previously described [22, 24]. BALF or peripheral blood was incubated with antibody-conjugated microbeads specific for the following markers: CD3 (T cells), CD15 (neutrophils), CD19 (B cells), CD235a (red blood cells), CD294 (eosinophils, basophils), and CD326 (epithelial cells). We did not use antibodies for CD294 (eosinophils) or CD326 (epithelial cells) for the blood samples because mononuclear cells were isolated from whole blood before antibody incubation via polyester gel centrifugation.

MMP28 Gene Expression

We extracted RNA from isolated cells, assessed it for purity, and then reverse transcribed it into cDNA with a High-Capacity cDNA Archive kit (Applied Biosystems). RT-PCR was performed per the manufacturer's instructions using HPRT and MMP28 (Hs01020031_m1) primer probe sets from Applied Biosystems. Primers and TaqMan probes (FAM dye-labeled) for MMP28 and HPRT were added to cDNA. Product amplification was measured with an ABI HT7900 Fast RT-PCR system (Applied Biosystems). The threshold cycle (Ct) was obtained from duplicate samples and averaged. The ΔCt was the difference between the average Ct for the target gene and the housekeeping gene (HPRT). The $\Delta\Delta$Ct was the ΔCt for a given sample minus the ΔCt of reference sample (set as the lowest expressing AM sample). The data are expressed as relative quantification (RQ) calculated as $2^{\Delta\Delta Ct}$ or $\log_2$-transformed RQ.

MMP28 BALF and Plasma Concentrations

BALF or plasma MMP28 collected on Day 1 was measured using an ELISA (Cat #: LS-F12061) specific for human MMP28 per the manufacturer's instructions (LifeSpan Biosciences). BALF and plasma samples were diluted 1:10 prior to running the ELISA. The lower limit of detection (LLOD) for each ELISA plate was designated as the value where the standard curve slope approached zero. Specimens with an MMP28 concentration below the LLOD were assigned an MMP28 concentration 50% the LLOD for analytical purposes.

BALF Measurements

Total protein concentration was determined using the bicinchoninic acid protein assay (ThermoFischer). BAL neutrophil percentages were calculated from manual inspection of cytospin preparations made from each BALF cell pellet. BALF IL-8, IL-6, and monocyte chemoattractant protein 1 (MCP-1) concentrations were measured using cytometric bead-based immunoassays (R and D Systems). BALF IL-17A concentrations were measured using a chemiluminescence immunoassay (Meso Scale Discovery).

Analyses

AM- and PBM-specific MMP28 gene expression (RQ), BALF and plasma MMP28 concentration, BALF cytokines (IL-8, IL-6, MCP-1, IL-17A), and total protein concentrations were all analyzed after $\log_2$ transformations to approach a normal distribution. The primary outcome of VFDs are defined as the number of days a subject is alive and free from mechanical ventilation between day 1 and day 28 after enrollment [25]. If a subject died before day 28, they were considered to have VFDs = 0. VFDs were divided by the median for analysis (VFDs = 14) (Figure E1). P_aO_2/F_iO_2 ratios (P/F ratios) were divided into mild-moderate (P/F > 150) vs. moderate-severe (P/F < 150) based on a recent classification of ARDS severity [26]. The highest sequential organ failure assessment (SOFA) score [27] within the first 7 days of ICU admission was expressed as a continuous variable or divided into tertiles. Alveolar neutrophil percentages (% PMNs) were divided into % PMNs ≤ 50% vs. % PMNs > 50%. Total protein concentration was divided by the median.

Our primary analysis was to test for an association between AM MMP28 gene expression and VFDs. Our explanatory analyses were to test for associations between AM MMP28 gene expression or alveolar MMP28 concentrations and P/F ratio, SOFA score, % PMNs, alveolar total protein concentration, and alveolar cytokine/chemokine concentrations (IL-8, IL-6, IL-17A, and MCP-1). The distribution of $\log_2$ AM MMP28 RQ, $\log_2$ PBM MMP28 RQ, and $\log_2$ plasma MMP28 concentrations were all approximately normal (Figure E1) and were analyzed using parametric tests. We used an unpaired t-test to test for significant differences between two groups. We used linear regression to test for an association between AM- or PBM-specific MMP28 gene expression and SOFA score. The distribution of $\log_2$ BALF MMP28 concentrations were right-skewed (Figure E1) and were analyzed using non-parametric tests. We used a Wilcoxon rank test to test for significant differences between two groups and a Kruskal-Wallis test to test for variance between three groups. We used a Pearson's test to identify correlations between AM- and PBM-specific MMP28 gene expression. We used a repeated measures ANOVA to test for variance between AM- and PBM-specific MMP28 expression over the first 8 days after ARDS onset in serially-sampled subjects. For all analyses, $p < 0.05$ was considered statistically significant. All analyses were conducted in GraphPad Prism version 7 or R version 3.5.1.

Results

Study Population

Subject characteristics are shown in Table 1. The subjects were predominantly male and sepsis was the most common ARDS risk factor. The mean P/F ratio was 156. All subjects met the revised 2012 Berlin Definition for ARDS [1].

Table 1
Subject Characteristics

Characteristic	ARDS Cohort (n = 76)
Demographic	
Age (mean ± SD)	50 ± 16
Sex (M/F)	45/31
Comorbidities	
Diabetes	16 (22%)
Cirrhosis	5 (7%)
Chronic renal insufficiency	2 (3%)
ARDS Risk Factor*, n (%)	
Sepsis	48 (64%)
Pneumonia	31 (41%)
Trauma	27 (37%)
Other	8 (11%)
Physiologic	
P/F Ratio (median, IQR)	156, (121–205)
APACHE II (mean ± SD)	22 ± 7
Outcome	
VFDs (median, IQR)	14, (0–21)
Mortality (28-day) (n, %)	11, 20%

* ARDS risk factors are not mutually exclusive; APACHE: Acute Physiology, Age, Chronic Health Evaluation; ARDS: acute respiratory distress syndrome; P/F ratio: P_aO_2/F_iO_2 ratio; SD: standard deviation; VFDs: ventilator-free days

AM MMP28 Gene Expression is Associated with Clinical Outcomes in ARDS

Our primary analysis was to test for an association between AM-specific MMP28 gene expression within 48 hours of ARDS onset and VFDs. Increased AM MMP28 gene expression on Day 1 was associated with worse VFDs (VFDs < 14 vs. VFDs ≥ 14, Fig. 1A). We next tested whether AM MMP28 gene expression on Day 1 was associated with P/F ratio to determine whether there was a link between AM MMP28 gene

expression and a lung-specific endpoint. Increased AM MMP28 gene expression was associated with worse P/F ratio (Fig. 1B). Notably, AM MMP28 gene expression was not associated with overall SOFA scores (Fig. 1C).

BALF MMP28 Concentrations are Associated with Clinical Outcomes in ARDS

In explanatory analyses, we next measured Day 1 soluble MMP28 concentrations from the BALF of subjects with ARDS and tested for associations between MMP28 and VFDs, P/F ratio, and SOFA in subjects with ARDS. There was no association between Day 1 BALF MMP28 concentration and VFDs (Fig. 2A). We found that increased BALF concentrations of MMP28 on Day 1 were associated with worse P/F ratio (Fig. 2B). There was no association between Day 1 BALF MMP28 concentration and highest SOFA score within 7 days after ARDS onset (Fig. 2C).

Associations Between MMP28 and Measures of Lung Inflammation and Injury

Alveolar capillary leak and alveolar neutrophil recruitment are assumed to be in the causal pathway for ARDS severity [4]. We next tested whether there was a relationship between Day 1 AM MMP28 expression or Day 1 BALF MMP28 concentration and physiologic measures of ARDS severity. AM MMP28 expression was not associated with either increased % PMNs or total protein in patients with ARDS (Fig. 3A and 3B). However, there was a significant association between increased BALF MMP28 concentrations and increased % PMNs and total protein (Fig. 3C and 3D).

MMPs have been shown to activate, inactivate, or modify the bioavailability of key cytokines and chemokines involved in ARDS pathogenesis [5, 9]. Therefore, we next tested whether Day 1 BALF MMP28 concentrations were associated with alveolar IL-8, IL-6, IL-17A, or MCP-1 concentrations in subjects with ARDS. IL-8, IL-17A, and MCP-1 are key chemokines involved in ARDS that recruit neutrophils and monocytes to the alveolar space [4]. IL-6 is a pleiotropic cytokine that promotes a wide range of local and systemic responses in ARDS. Increased BALF MMP28 concentrations were significantly associated with increased BALF IL-6, IL-17A, and MCP-1 concentrations (Fig. 4).

Human Myeloid Cell Expression of MMP28 in ARDS

We next tested whether MMP28 was differentially expressed in paired AMs vs. PBMs simultaneously collected from individual subjects with ARDS. There was a significant correlation between the relative AM and PBM MMP28 gene expression ($r = 0.61$, $p < 0.01$, Fig. 5A). We then analyzed serial AM or PBM MMP28 gene expression in subjects who were alive and remained on mechanical ventilation for at least 8 days. AM and PBM MMP28 gene expression persisted over the course of the first 8 days after ARDS onset in subjects who were alive and remained on mechanical ventilation (Fig. 5B and 5C). Increased PBM MMP28 expression on Day 1 was associated with worse P/F ratio, however was not associated with VFDs or overall SOFA scores (Figure E2). Plasma MMP28 concentrations measured within 48 hours of ARDS onset were not associated with any clinical outcomes (Figure E3).

Discussion

The major findings of our study are that increased AM MMP28 gene expression and increased BALF MMP28 concentrations at the time of ARDS onset are associated with worse ARDS clinical outcomes. There is emerging interest in understanding how AM polarization may orchestrate the inflammatory and reparative processes that occur during ARDS [4, 21]. In animal models of Pseudomonas aeruginosa lung infection, MMP28 plays a key role in macrophage chemotaxis [19] and in modulating macrophage polarity [12]. Our study is the first in humans to demonstrate that increased AM MMP28 gene expression within the first 48 hours after ARDS onset is associated with worse VFDs (Fig. 1). This finding may be explained in part by our additional findings that increased AM MMP28 gene expression is associated with worse oxygenation and alveolar MMP28 levels are associated with increased % PMNs (Fig. 3) and alveolar inflammatory cytokine/chemokine concentrations (Fig. 4).

A strength of our study comes from our highly unique sample set of BALF and blood from well-characterized cases of ARDS. We measured AM- and PBM-specific MMP28 expression as well as soluble MMP28 concentrations from BALF and plasma from subjects enrolled in a phase-II ARDS clinical trial. Importantly, we found that both AMs and PBMs express MMP28 during ARDS (Fig. 5) and that MMP28 gene expression persists in subjects who remain alive and mechanically ventilated for at least 8 days after ARDS onset. AM and PBM MMP28 expression were correlated in subjects with paired BALF and blood samples (Fig. 5A), however PBM MMP28 expression and plasma MMP28 concentrations were not associated with important ARDS outcomes (Figures E2 and E3). These findings suggest that the bioactivity of MMP28 in the lung may be more relevant to clinical outcomes in patients with ARDS than the bioactivity of MMP28 in the systemic compartment.

This study demonstrated a consistent association between alveolar MMP28 and ARDS outcomes. However, the mechanistic role MMP28 may play in this association is unclear. In animal models of acute lung injury, $Mmp28^{-/-}$ mice have decreased BALF and lung homogenate concentrations of KC [19], which is the functional homologue of the human neutrophil chemokine IL-8. Although we found that increased MMP28 BALF concentrations were associated with increased alveolar % neutrophils (Fig. 3), we did not find an association between MMP28 and BALF IL-8 concentrations (Fig. 4). It is possible that MMP28 modulates alveolar inflammation by binding, retaining, or concentrating other mediators such as IL-6, IL-17A, or MCP-1 (Fig. 4). It is also possible that MMP28 levels may simply be related to overall illness severity. However, both AM MMP28 gene expression and BALF MMP28 concentrations were not associated with overall organ dysfunction (SOFA scores, Figs. 1 and 2). These findings imply that MMP28 might be related to ARDS outcomes via lung-specific mechanisms and not simply overall severity of illness. Lastly, the lung epithelium is another potential source of BAL MMP28 [16, 20, 28], and elevated levels in the BALF may reflect increased secretion or release from injured epithelium.

Our study has several limitations. First, our findings were derived from a single cohort and will need to be replicated in future ARDS studies that employ alveolar sampling. Second, we measured transcript levels averaged across all AM populations. It is possible that different AM subtypes (e.g. resident vs. recruited

AMs) [29–32] have distinct MMP28 expression patterns and different associations with clinical outcomes. Finally, we did not have a control group of mechanically ventilated patients without ARDS. Thus, our finding associating increased AM MMP28 expression and BALF MMP28 concentration with worse outcomes might apply to patients with acute respiratory failure in general and not be specific to ARDS.

Conclusions

There are currently no therapies directed at the underlying pathophysiology of ARDS that have been shown to improve patient outcomes [33]. MMP28 represents a novel target that could potentially be leveraged to alter the host immune response in ARDS. Our study is the first to report that increased AM-specific MMP28 expression and increased BALF MMP28 concentrations are associated with worse ARDS outcomes. PBM-specific MMP28 expression and plasma MMP28 concentration are not associated with ARDS outcomes. Further work is needed to determine whether the associations between AM MMP28 and BALF MMP28 concentrations and alveolar inflammatory mediators are mechanistically linked.

Abbreviations

AM:alveolar macrophage; ARDS:acute respiratory distress syndrome; BALF:bronchoalveolar lavage fluid; CI:confidence interval; Ct:threshold cycle; ELISA:enzyme-linked immunosorbent assay; LLOD:lower limit of detection; MCP-1:monocyte chemoattractant protein 1; MMP:matrix metalloproteinases; PBM:peripheral blood monocyte; P/F ratio:P_aO_2/F_iO_2 ratio; RQ:relative quantification; VFD:ventilator-free days

Declarations

Ethics Approval and Consent to Participate: All studies were approved by the Human Subjects Division at the University of Washington.

Consent for Publication: Not applicable

Availability of Data and Materials: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests: The authors declare that they have no competing interests.

Funding: NIH NHLBI P50 HL073996 (Dr. Wurfel), NIH K23 HL144916 and Francis Family Foundation/Parker B. Francis Fellowship (Dr. Morrell), NIH K23 HL120896 (Dr. Mikacenic), NIH R01 HL116514 (Dr. Manicone)

Author's Contributions: A.M.M. contributed to the conception of the work. E.D.M., M.M.W., C.M., and A.M.M. contributed to the design of the work. E.D.M., K.G., S.K., R.D.S., M.M.W., C.M., and A.M.M.

contributed to the acquisition, analysis, and interpretation of the data for the work. E.D.M., C.M., M.M.W., and A.M.M drafted and revised the manuscript for important intellectual content. E.D.M., K.G., S.K., R.D.S., M.M.W., C.M., and A.M.M. significantly contributed to and approved the final version of the manuscript for publication. E.D.M., K.G., S.K., R.D.S., M.M.W., C.M., and A.M.M. agree to be accountable for all aspects of the work.

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Additional Files

File name: CC_MMP28_supplement_edm_1.2.20

File format: .docx

Title of data: Supplemental Results

Description of data: word file containing the supplemental figures (Figure E1, E2, and E3)

Figures

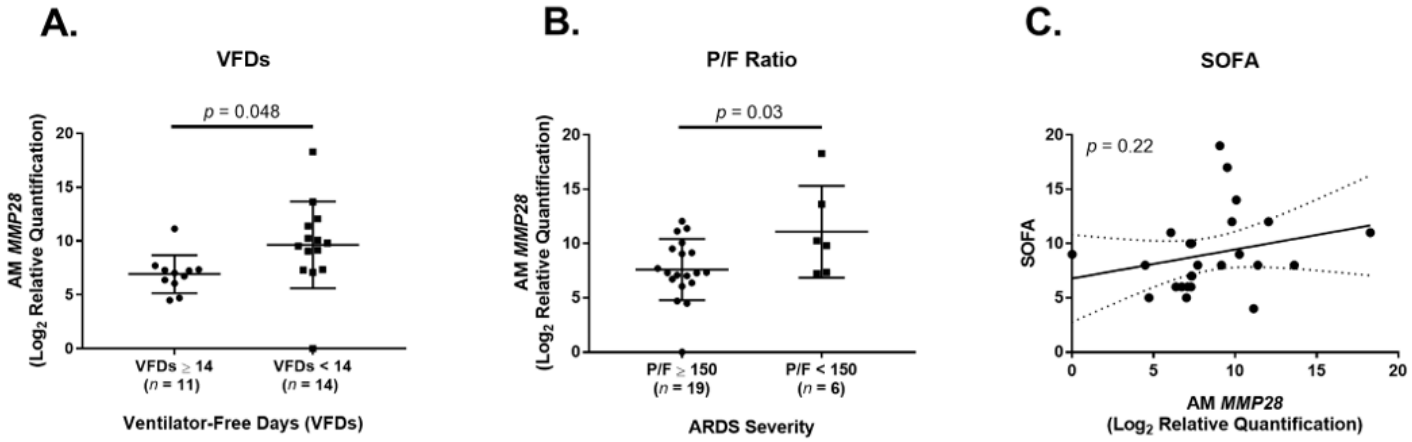

Figure 1

Alveolar Macrophage (AM) MMP28 Gene Expression is Associated with Clinical Outcomes in Acute Respiratory Distress Syndrome (ARDS). RNA was purified from negatively-selected AMs collected from BALF from subjects with ARDS. A) AM-specific relative gene expression of MMP28 was higher in subjects with worse ventilator-free days (VFDs) (VFDs < 14) vs. better VFDs (VFDs ≥ 14) (p = 0.048, unpaired t-test). Subjects were divided by the median VFDs (VFD = 14, Figure E1). Shown are the individual values, mean, and standard deviation. B) AM-specific relative gene expression of MMP28 was higher in subjects with a P/F ratio < 150 vs. P/F ratio ≥ 150 (p = 0.03, unpaired t-test). Shown are the individual values, mean, and standard deviation. C) AM-specific relative gene expression of MMP28 was not associated with worst SOFA score over the first 7 days of hospitalization (p = 0.22 testing whether the slope of the relationship between AM MMP28 relative expression and SOFA scores deviates from zero, simple linear regression). Shown are the individual values, best-fit linear regression line, and 95% confidence interval.

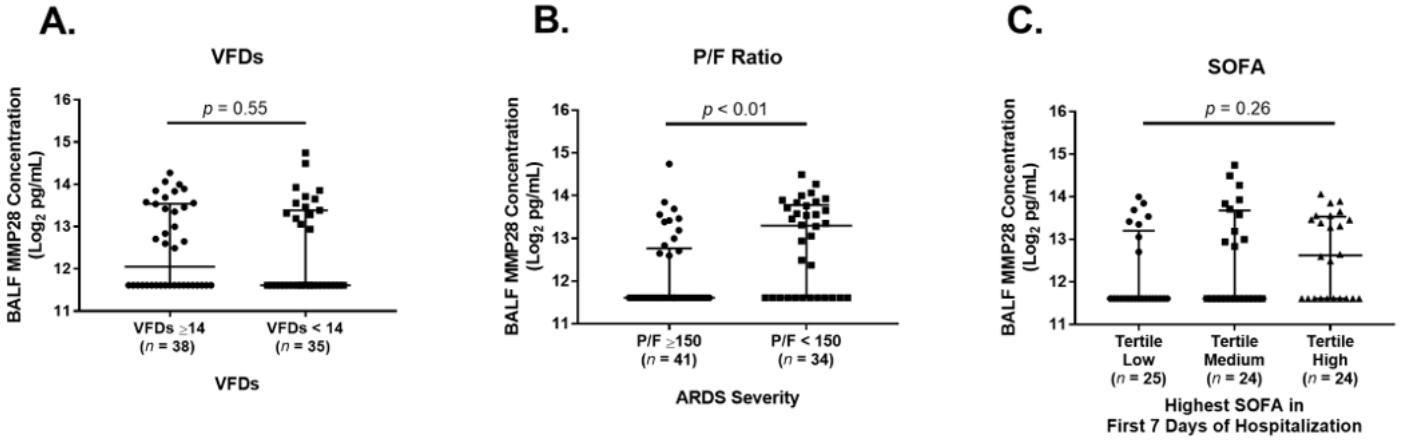

Figure 2

Associations Between Bronchoalveolar Lavage Fluid (BALF) MMP28 Concentrations and Clinical Outcomes. We used an ELISA to measure MMP28 concentrations from BALF collected from subjects with ARDS. A) BALF MMP28 concentrations were not different in subjects with worse ventilator-free days (VFDs) (VFDs < 14) vs. better VFDs (VFDs ≥ 14) (p = 0.55, Wilcoxon rank test). Shown are the individual values, median, and interquartile range. B) BALF MMP28 concentrations were higher in subjects with a P/F ratio < 150 vs. P/F ratio ≥ 150 (p < 0.01, Wilcoxon rank test). Shown are the individual values, median, and interquartile range. C) BALF MMP28 concentrations were not associated with tertiles of highest SOFA score within the first 7 days of hospitalization (Tertile Low = lowest SOFA scores; Tertile High = highest SOFA scores (p = 0.26, Kruskal-Wallis test for variance). Shown are the individual values, median, and interquartile range.

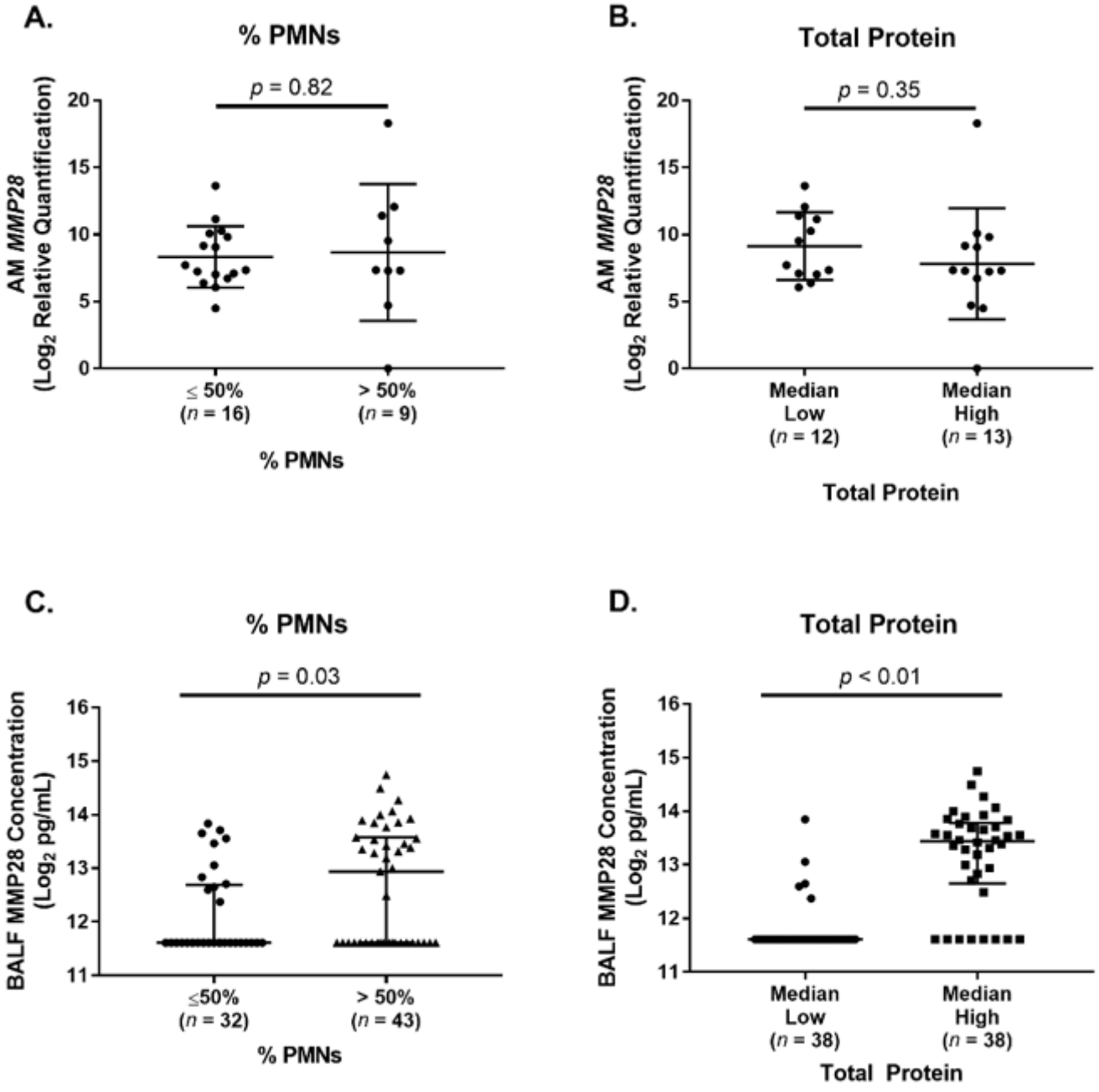

Figure 3

Associations Between MMP28 and Measures of Lung Inflammation and Injury. A) AM-specific MMP28 gene expression was not different in subjects % PMNs ≤ 50% vs. subjects with % PMNs > 50% (p = 0.82, unpaired t-test). Shown are the individual values, mean, and standard deviation. B) AM-specific MMP28 relative gene exprssion was not different in subject with higher alveolar total protein vs. subjects with lower alveolar total protein (p = 0.35, unpaired t-test). Subjects were divided by the median alveolar total protein concentration (194.8 µg/mL). Shown are the individual values, mean, and standard deviation. C) BALF MMP28 concentrations were lower in subjects with % PMNs ≤ 50% vs. subjects with % PMNs > 50% (p = 0.03, Wilcoxon rank test). Shown are the individual values, median, and interquartile range. D) BALF MMP28 concentrations were lower in subjects with lower alveolar total protein vs. subjects with higher total protein (p < 0.01, Wilcoxon rank test). Subjects were divided by the median alveolar total protein concentration (306.5 µg/mL). Shown are the individual values, median, and interquartile range.

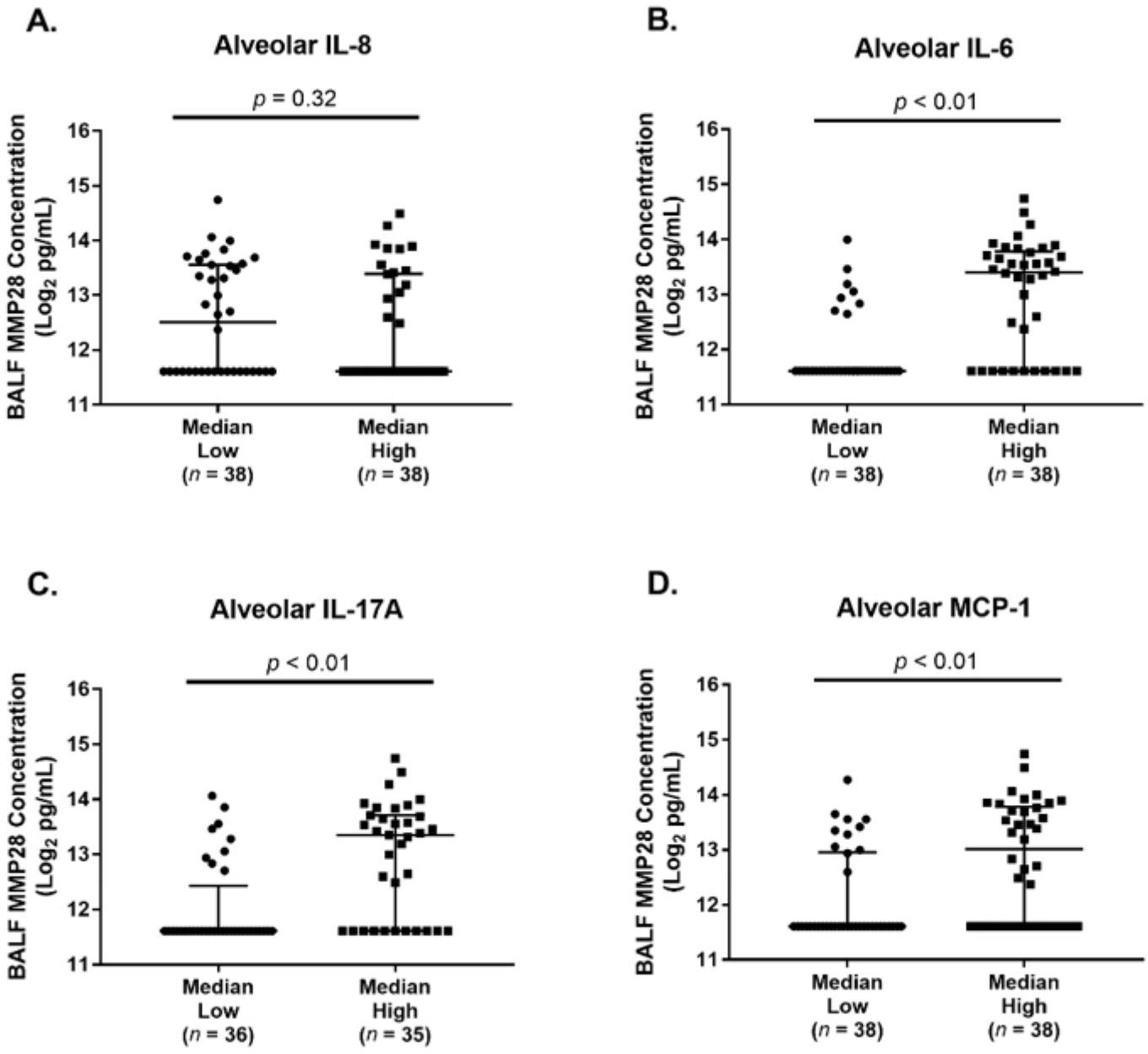

Figure 4

BALF MMP28 Concentrations are Associated with Alveolar Cytokine/Chemokine Concentrations. A) BALF MMP28 concentrations were not different subjects with lower IL-8 vs. higher IL-8 alveolar concentrations (p = 0.32, Wilcoxon rank test). Subjects were divided by the median alveolar IL-8 concentration (479.6 pg/mL). Shown are the individual values, median, and interquartile range. B) BALF MMP28 concentrations were lower in subjects with lower IL-6 vs. higher IL-6 alveolar concentrations (p < 0.01, Wilcoxon rank test). Subjects were divided by the median alveolar IL-6 concentration (197.3 pg/mL). Shown are the individual values, median, and interquartile range. C) BALF MMP28 concentrations were lower in subjects with lower IL-17A vs. higher IL-17A alveolar concentrations (p < 0.01, Wilcoxon rank test). Subjects were divided by the median alveolar IL-17A concentration (0.6 pg/mL). Shown are the

individual values, median, and interquartile range. D) BALF MMP28 concentrations were lower in subjects with lower MCP-1 vs. higher MCP-1 alveolar concentrations ($p < 0.01$, Wilcoxon rank test). Subjects were divided by the median alveolar MCP-1 concentration (524.3 pg/mL). Shown are the individual values, median, and interquartile range.

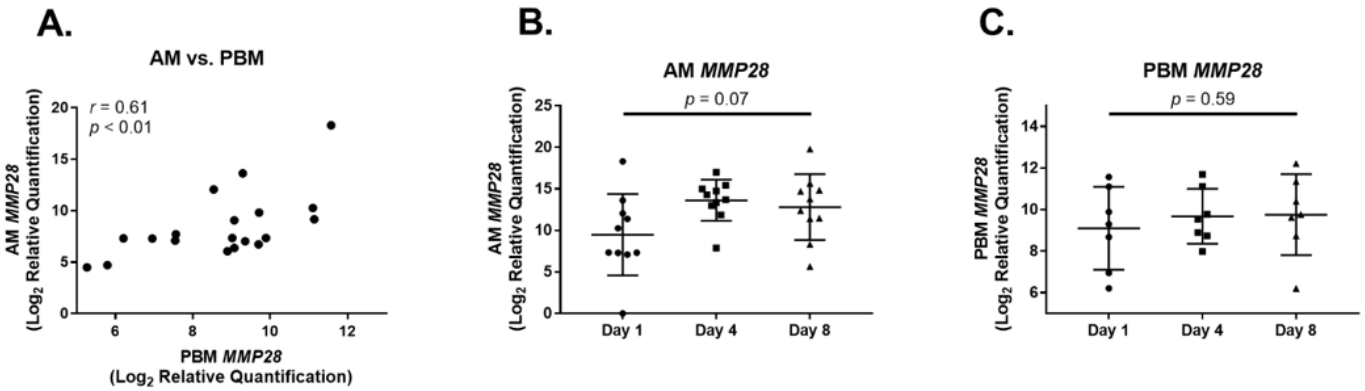

Figure 5

AMs and PBMs Have Similar Relative Expression of MMP28 in Subjects with ARDS. RNA was purified from negatively-selected AMs and PBMs collected from BALF and blood, respectively, from subjects with ARDS. A) AM- and PBM-specific MMP28 gene epression are correlated in subjects with ARDS ($r = 0.61$, $p < 0.01$, Pearson's test). Shown are the individual values. B) AM MMP28 gene expression persists in subjects alive and on mechanical ventilation for at least 8 days after ARDS onset ($p = 0.07$, repeated measures ANOVA). Shown are individual values, mean, and standard deviation. D) PBM MMP28 expression persists in subjects alive and on mechanical ventilation for at least 8 days after ARDS onset ($p = 0.59$, repeated measures ANOVA). Shown are individual values, mean, and standard deviation.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- CCMMP28supplementedm1.2.20.docx