Title: **Early Plasma Matrix Metalloproteinase Profiles: A Novel Pathway in Pediatric Acute Respiratory Distress Syndrome**

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ONLINE DATA SUPPLEMENT

SUPPLEMENTAL DATA

Supplemental Methods

In order to identify potential latent classes in the derivation group, we fit multiple finite mixture models using $\log_{10}$-transformed MMP-1, -3, -7, -8, and -9, and TIMP-1 and -2 measurements, selected the best-fitting model, and assigned patients to groups based on the estimated probability of latent class membership (Mplus Version 7, Muthen & Muthen). Missing data were handled using the Full Information Maximum Likelihood (FIML) approach, which does not require exclusion of patients with missing data. To satisfy the condition of local independence, MMP-2 and active MMP-9 were excluded as clustering variables due to collinearity with TIMP-2 and total MMP-9, respectively. The optimal number of patient groups was selected using according to the minimal Bayesian Information Criteria, maximal entropy, and the Lo-Mendell-Rubin (LMR) Adjusted Likelihood Ratio Test. To avoid local minima, we used 500 random starts and required that all converge on the same loglikelihood values. The classification fit of the proposed groups was tested by comparing the mean estimated probability of group membership among each proposed group. The latent MMP profile of each group was then described by comparing the mean levels of each MMP pathway biomarker, adjusted for the probability of group membership.

To determine whether latent profile membership could be modeled with linear regression, we fit a linear regression associating the probability of latent profile membership with individual MMP measurements. Least angle regression was used to select the order of addition of MMPs to the model, and the likelihood ratio test was used to limit the size of the model. MMP measurements

from the validation cohort were then entered into the linear regression in order to estimate the probability of group membership in the validation cohort. The validation cohort was then independently separated into latent groups using Mplus, and the assignments from each method were compared.

SUPPLEMENTAL DATA 1:

Relationships Between Individual MMP Measurements and Clinical Characteristics

Table E1) Characteristics of Enrolled Patients with vs. without MMP Pathway Measurements

Characteristics	**All patients** (n=326)	**With MMP Measurements** (n=235)	**Without MMP Measurements** (n=91)	**Significance**
Age (median years, IQR)	4.5 (1.0-11.5)	4.1 (1.0-11.5)	4.7 (1.0-11.5)	p=0.801
Sex (male n, %)	177 (54.3)	125 (53.2)	52 (57.1)	p=0.521
Race (n, %)				**p=0.007**
White	203 (62.3)	160 (68.1)	43 (47.3)	
Unknown	44 (13.5)	30 (12.8)	14 (15.4)	
Black	28 (8.6)	17 (7.2)	11 (12.1)	
Asian/PI	24 (7.4)	15 (6.4)	9 (9.9)	
Multiple	24 (7.4)	12 (5.1)	12 (13.2)	
American Indian	3 (0.9)	1 (0.4)	2 (2.2)	
Ethnicity (n, %)				p=0.435
Hispanic/Latino	123 (37.7)	84 (35.7)	39 (42.9)	
Not Hispanic/Latino	188 (57.7)	139 (59.2)	39 (53.9)	
Unknown	15 (4.6)	12 (5.1)	3 (3.3)	
Lung Injury Etiology (n, %)				p=0.361
Pneumonia	183 (56.8)	127 (54.5)	56 (62.9)	
Sepsis	67 (20.8)	49 (21.0)	18 (20.2)	
Other	37 (11.5)	27 (11.6)	10 (11.2)	
Trauma	17 (5.3)	13 (5.6)	4 (4.5)	
Aspiration	13 (4.0)	12 (5.2)	1 (1.1)	
TRALI	5 (1.6)	5 (2.2)	0 (0.0)	
Cancer/HCT (n, %)				p=0.491
Cancer	36 (11.1)	26 (11.2)	10 (11.0)	
HCT	32 (9.9)	29 (12.6)	3 (3.3)	
Cancer or HCT	54 (16.6)	41 (17.5)	13 (14.3)	
WBC (median, IQR)	8.2 (4.4-14.3)	7.8 (4.3-14)	10.4 (4.8-15.7)	p=0.247
Day 1 Illness Severity (median, IQR)				
PaO_2/FiO_2 Ratio (P/F)	128.3 (82.9-210)	132 (84.3-220)	121.9 (80.6-194.3)	p=0.440
Oxygenation Index (OI)	10.3 (5.9-19.7)	10.1 (6.1-19.6)	12.1 (5.6-21.1)	p=0.609
PRISM-3	12 (7-20)	12 (7-20)	12 (8-20)	p=0.488
Day 3 Illness Severity (median, IQR)				
PaO_2/FiO_2 Ratio (P/F)	166.5 (107.2-241.4)	173.9 (116.1-264.8)	141.7 (100-209.2)	**p=0.017**
Oxygenation Index (OI)	8.2 (4.8-15.3)	7.9 (4.5-14.5)	9.8 (5.6-20)	p=0.061
PELOD	11 (1-12)	11 (1-12)	11 (1-12)	p=0.905

Legend: Associations tested with Fisher exact test for categorical variables and Wilcoxon rank sum for non-normally distributed continuous variables. On day 1, P/F ratio n=299; OI n=272; PRISM-3 n=275. On day 3, P/F ratio n=284; OI n=244, PELOD n=326.

Table E2) Plasma MMP Levels Are Associated with Survivor Morbidity

Biomarker (ng/mL)	**Survivor PELOD** (n=193)	
MMP-1	0.291	**p<0.001**
MMP-2	0.278	**p<0.001**
MMP-3	0.220	**p=0.004**
MMP-7	0.212	**p=0.007**
MMP-8	0.124	p=0.120
MMP-9	-0.104	p=0.179
MMP-9 (active)	-0.090	p=0.262
TIMP-1	0.427	**p<0.001**
TIMP-2	0.285	**p<0.001**
MMP-9/ TIMP-1	-0.335	**p<0.001**
MMP-9 (active)/ **TIMP-1**	-0.305	**p<0.001**

Legend: Associations were tested using Spearman's ρ with univariate significance.

Table E3) Plasma MMP Levels Are Associated with Day 1 Illness Severity

Biomarker (ng/mL)	**Day 1 P/F**		**Day 1 OI**		**Day 1 PRISM-3**	
MMP-1	-0.131	p=0.075	0.169	**p=0.029**	0.222	**p=0.004**
MMP-2	-0.006	p=0.935	0.005	p=0.948	0.167	**p=0.032**
MMP-3	-0.051	p=0.479	0.106	p=0.162	0.350	**p<0.001**
MMP-7	-0.121	p=0.100	0.191	**p=0.013**	0.184	**p=0.018**
MMP-8	-0.147	**p=0.045**	0.208	**p=0.007**	0.152	p=0.051
MMP-9	-0.124	p=0.087	0.067	p=0.380	-0.187	**p=0.012**
aMMP-9	-0.027	p=0.711	-0.057	p=0.462	-0.215	**p=0.005**
TIMP-1	-0.235	**p=0.001**	0.333	**p<0.001**	0.451	**p<0.001**
TIMP-2	-0.070	p=0.340	0.091	p=0.243	0.238	**p=0.002**
MMP-9 / TIMP-1	0.070	p=0.332	-0.164	**p=0.030**	-0.413	**p<0.001**
aMMP-9 / TIMP-1	0.200	**p=0.011**	-0.283	**p<0.001**	-0.438	**p<0.001**

Legend: Associations were tested using Spearman's ρ with univariate significance.

Table E4) Plasma MMP Levels Are Associated with Day 3 Illness Severity

Biomarker (ng/mL)	**Day 3 P/F**		**Day 3 OI**		**Day 3 PELOD**	
MMP-1	-0.073	p=0.338	0.124	p=0.124	0.263	**p<0.001**
MMP-2	0.009	p=0.902	0.004	p=0.958	0.212	**p=0.003**
MMP-3	-0.048	p=0.515	0.034	p=0.668	0.195	**p=0.005**
MMP-7	-0.162	**p=0.032**	0.119	p=0.139	0.273	**p<0.001**
MMP-8	-0.048	p=0.532	0.034	p=0.674	0.179	**p=0.012**
MMP-9	0.058	p=0.437	-0.077	p=0.338	-0.153	**p=0.028**
aMMP-9	0.100	p=0.190	-0.154	p=0.057	-0.148	**p=0.038**
TIMP-1	-0.205	**p=0.005**	0.239	**p=0.003**	0.304	**p<0.001**
TIMP-2	-0.060	p=0.432	0.056	p=0.490	0.236	**p<0.001**
MMP-9/ TIMP-1	0.145	**p=0.049**	-0.178	**p=0.025**	-0.293	**p<0.001**
aMMP-9 / TIMP-1	0.186	**p=0.022**	-0.236	**p=0.006**	-0.275	**p<0.001**

Legend: Associations were tested using Spearman's ρ with univariate significance.

Table E5) Plasma MMP Levels Are Associated with Indirect Lung Injury

Biomarker (ng/mL)	**Direct Lung Injury** (n=140)		**Indirect Lung Injury** (n=93)		**Significance**	**Adjusted Significance**
MMP-1	1092.4	(606.6-2292.5)	1196.5	(757.9-2677.1)	p=0.197	p=0.317
MMP-2	487.4	(350.6-704.1)	492	(361.9-727.7)	p=0.623	p=0.858
MMP-3	3	(1.8-7.2)	4.9	(2.4-10)	**p=0.014**	p=0.130
MMP-7	393.3	(157.2-886.4)	521.5	(230.8-1280.5)	**p=0.031**	p=0.773
MMP-8	21.5	(11.7-48.6)	40.3	(17.1-89.0)	**p=0.032**	p=0.250
MMP-9	104	(61-231)	119	(61-248)	p=0.501	p=0.084
MMP-9 (active)	232.1	(127.1-468.4)	202.5	(107.5-515)	p=0.691	p=0.394
TIMP-1	274.5	(139-487)	377.5	(189-766)	**p=0.018**	**p=0.042**
TIMP-2	77.8	(57.9-96.2)	81.8	(67.9-102.9)	p=0.234	p=0.585
MMP-9/ TIMP-1	0.44	(0.16-1.11)	0.34	(0.11-0.83)	p=0.239	p=0.185
MMP-9 (active)/ **TIMP-1**	0.83	(0.35-2.14)	0.62	(0.18-1.38)	p=0.072	p=0.352

Legend: Median and interquartile range of plasma MMP pathway proteins are shown for pediatric ARDS patients with direct (n=140, pneumonia or aspiration) and indirect lung injury (n=93, non-pulmonary sepsis, transfusion related acute lung injury, trauama, and other). Significance was tested using the Wilcoxon rank sum test. Adjusted significance was tested using linear regression for outcome of $\log_{10}$-transformed biomarker as predicted by direct vs indirect lung injury status, with adjustment for cancer/HCT status and day 1 P/F ratio.

Table E6) Plasma MMP Levels Are Associated with Cancer/HCT

Biomarker (ng/mL)	**No Cancer/HCT** (n=194)		**Cancer/HCT** (n=41)		**Significance**	**Adjusted Significance**
MMP-1	1,096.7	(663.2-2,324.3)	1,618.1	(485.9-3,250.2)	p=0.385	p=0.532
MMP-2	491.3	(351.1-711.8)	582.6	(402.2-768.6)	p=0.287	p=0.377
MMP-3	3.5	(1.9-7.9)	3.9	(2.7-8.8)	p=0.194	p=0.458
MMP-7	400.8	(173.0-1026.2)	791.9	(304.2-2090.2)	**p=0.007**	**p=0.006**
MMP-8	31.4	(13.3-70.4)	28.4	(8.4-56.1)	p=0.363	p=0.373
MMP-9	129.0	(66-255)	61.0	(34-111)	**p<0.001**	**p=0.001**
MMP-9 (active)	243.9	(126.9-510.2)	131.6	(76.9-269.0)	**p<0.001**	**p<0.001**
TIMP-1	272.5	(138-483)	583.0	(340-972)	**p<0.001**	**p<0.001**
TIMP-2	78.8	(61.0-97.1)	83.3	(68.9-110.7)	p=0.347	p=0.558
MMP-9/ TIMP-1	0.50	(0.18-1.16)	0.09	(0.07-0.16)	**p<0.001**	**p<0.001**
MMP-9 (active)/ **TIMP-1**	0.93	(0.34-2.27)	0.20	(0.10-0.76)	**p<0.001**	**p<0.001**

Legend: Median and interquartile range of plasma MMP pathway proteins are shown for pediatric ARDS patients with prior Cancer/HCT (n=41) and without prior cancer/HCT (n=194). Significance was tested using the Wilcoxon rank sum test. Adjusted significance was tested using linear regression for outcome of $\log_{10}$-transformed biomarker as predicted by cancer/HCT status, with adjustment for age, sex, race, and WBC.

Table E7) Plasma MMP Levels Are Associated with WBC

Biomarker (ng/mL)	**WBC**		
	ρ	**Significance**	**Adjusted Significance**
MMP-1	-0.095	p=0.195	p=0.472
MMP-2	-0.192	**p=0.008**	p=0.181
MMP-3	-0.149	**p=0.037**	p=0.797
MMP-7	-0.104	p=0.157	p=0.566
MMP-8	0.000	p=0.997	p=0.091
MMP-9	0.378	**p<0.001**	**p=0.002**
MMP-9 (active)	0.431	**p<0.001**	**p<0.001**
TIMP-1	-0.304	**p<0.001**	p=0.550
TIMP-2	-0.151	**p=0.039**	p=0.808
MMP-9/ TIMP-1	0.471	**p<0.001**	p=0.076
MMP-9 (active)/ **TIMP-1**	0.467	**p<0.001**	**p=0.001**

Legend: Significance was tested using Spearman's ρ associations. Adjusted significance was tested using linear regression for the outcome of $\log_{10}$-transformed biomarker as predicted by WBC, with adjustment for age, sex, race, and cancer/HCT status.

SUPPLEMENTAL DATA 2

Derivation of Latent Classes

Table E8) Characteristics of Derivation vs. Validation Cohorts

Characteristics	**All patients** (n=235)	**Derivation** (n=126)	**Validation** (n=109)	**Significance**
Age (median years, IQR)	4.1 (1.0-11.5)	3.6 (1.0-11.4)	5.0 (1.1-11.9)	p=0.508
Sex (male n, %)	125 (53.2)	65 (51.6)	60 (55.1)	p=0.596
Race (n, %)				**p=0.018**
White	160 (68.1)	89 (70.6)	71 (65.1)	
Unknown	30 (12.8)	13 (10.3)	17 (15.6)	
Black	17 (7.2)	10 (7.9)	7 (6.4)	
Asian/PI	15 (6.4)	12 (9.5)	3 (2.8)	
Multiple	12 (5.1)	2 (1.6)	10 (9.2)	
American Indian	1 (0.4)	0 (0)	1 (0.9)	
Ethnicity (n, %)				p=0.387
Hispanic/Latino	84 (35.7)	40 (31.8)	44 (40.4)	
Not Hispanic/Latino	139 (59.2)	79 (62.7)	60 (55.1)	
Unknown	12 (5.1)	7 (5.6)	5 (4.6)	
Lung Injury Etiology (n, %)				**p=0.016**
Pneumonia	127 (54.5)	72 (58.1)	55 (50.5)	
Sepsis	49 (21.0)	23 (18.6)	26 (23.9)	
Other	27 (11.6)	18 (14.5)	9 (8.3)	
Trauma	13 (5.6)	2 (1.6)	11 (10.1)	
Aspiration	12 (5.2)	8 (6.5)	4 (3.7)	
TRALI	5 (2.2)	1 (0.8)	4 (3.7)	
Cancer/HCT (n, %)				
Cancer	26 (11.2)	16 (12.9)	10 (9.2)	p=0.367
HCT	29 (12.6)	18 (14.4)	11 (10.4)	p=0.358
Cancer or HCT	41 (17.5)	25 (19.8)	16 (14.7)	p=0.298
WBC (median, IQR)	7.8 (4.3-14)	7.8 (3.7-14.1)	7.7 (4.6-13.6)	p=0.776
Day 1 Illness Severity (median, IQR)				
PaO_2/FiO_2 Ratio (P/F)	132 (84.3-220)	148.8 (91.1-228.3)	125.9 (76-205.7)	p=0.138
Oxygenation Index (OI)	10.1 (6.1-19.6)	10.1 (6.1-18.3)	9.8 (5.7-21.6)	p=0.794
PRISM-3	12 (7-20)	12 (8-20)	12 (5-19)	p=0.250
Day 3 Illness Severity (median, IQR)				
PaO_2/FiO_2 Ratio (P/F)	173.9 (116.1-264.8)	173.3 (105.2-266.7)	174.5 (118.2-264.5)	p=0.727
Oxygenation Index (OI)	7.9 (4.5-14.5)	8.0 (4.4-14.5)	7.0 (4.9-15.2)	p=0.771
PELOD	11 (1-12)	21 (11-30)	21 (11-30)	p=0.740
Mortality	42 (17.9)	22 (17.5)	20 (18.4)	p=0.859
Mortality or Severe Morbidity	81 (34.5)	45 (35.7)	36 (33.0)	p=0.666

Legend: Associations tested with Fisher exact test for categorical variables and Wilcoxon rank sum for non-normally distributed continuous variables. On day 1, P/F ratio n=219; OI n=198; PRISM-3 n=198. On day 3, P/F ratio n=208; OI n=181, PELOD n=235.

Table E9) MMP Pathway Correlation Matrix

	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-9a	TIMP-1	TIMP-2
MMP-1	1								
MMP-2	**0.237 p<0.001**	1							
MMP-3	0.164 p=0.053	0.149 p=0.144	1						
MMP-7	**0.199 p=0.001**	0.150 p=0.061	**0.215 p=0.001**	1					
MMP-8	**0.214 p<0.001**	0.090 p=1.000	0.124 p=0.602	0.153 p=0.051	1				
MMP-9	0.042 p=1.000	-0.017 p=1.000	-0.025 p=1.000	-0.079 p=1.000	**0.242 p<0.001**	1			
MMP-9a	0.050 p=1.000	-0.041 p=1.000	-0.033 p=1.000	-0.047 p=1.000	**0.260 p<0.001**	**0.693 p<0.001**	1		
TIMP-1	**0.303 p<0.001**	**0.216 p=0.001**	**0.380 p<0.001**	**0.274 p<0.001**	**0.247 p<0.001**	-0.068 p=1.000	-0.097 p=1.000	1	
TIMP-2	**0.246 p<0.001**	**0.710 p<0.001**	**0.212 p=0.001**	**0.200 p=0.001**	**0.167 p=0.017**	0.013 p=1.000	0.015 p=1.000	**0.303 p<0.001**	1

Legend: Kendall's rank correlation (tau coefficient) with Bonferroni-adjusted p-values. Bolded black values indicate statistically significant weak correlation (tau <0.4). Bolded red values indicate statistically significant moderate-to-strong correlation (tau ≥0.4).

PEDIATRIC ARDS DERIVATION COHORT (n=126)

Table E10a) MMP Latent Profile Analysis in Pediatric ARDS Derivation Cohort

Models	AIC	BIC	ABIC	k-1 LMR	Entropy	Profile Size
2 profiles	787.287	858.194	779.136	**p=0.002**	0.829	n=41,85
3 profiles	737.522	833.956	727.437	p=0.153	0.771	n=39,39,48
4 profiles	686.174	808.134	672.155	p=0.167	0.810	n=9,32,39,46

Legend: Akaike, Bayesian, and sample-size adjusted Bayesian Information Criteria (AIC, BIC, ABIC, respectively) are listed for 2, 3, and 4 profile models. Each model was compared to the model with 1 fewer latent profile (k-1) using the Lo-Mendell-Rubin (LMR) Adjusted Likelihood Ratio Test. Profile Size refers to sample size of each Latent Profile according to most likely assignment based on posterior probabilities. Although a 3 class model had a smaller BIC relative to the 2 profile model, it was not significantly different by the LMR test (p=0.153) and had lower entropy and therefore was determined to be overfit and a 2 profile model was selected.

Table E10b) Average Latent Class Probabilities for Most Likely Latent Class Membership (Row) by Latent Class (Column) in the Derivation Cohort

	Probability of Profile 1	Probability of Profile 2
Patients Most Likely to belong to Profile 1 (n=41)	94.9%	5.1%
Patients Most Likely to belong to Profile 2 (n=85)	5.1%	94.9%

Legend: Patients were assigned to latent profiles based on the maximum posterior probability for profile 1 vs. 2 (rows). The mean probability of being in profile 1 vs. profile 2 is listed for patients whose maximum posterior probability grouped them in profile 1 and again for patients whose maximum posterior probability grouped them in profile 2. These data show that misclassification in the 2-profile model was low.

Table E10c) Incidence and Mortality of Latent Profile 1 vs. 2 in the Derivation Cohort

	Profile 1	Profile 2	Comparison
Number of Patients			
Maximum Posterior Probability	41 (32.5%)	85 (67.5%)	n/a
Estimated Profile Counts	43.3 (34.4%)	82.7 (65.6%)	n/a
Mortality			
Maximum Posterior Probability	11 (26.8%)	11 (13.1%)	p=0.058
Estimated Profile Counts	11.6 (26.7%)	10.5 (12.7%)	**p=0.047**

Legend: Profile counts were determined by maximum posterior probability or estimated according to each patient's probability of membership in each profile. Mortality counts were compared by Pearson chi-squared test when profiles were assigned by maximum posterior probability or by fitted regression estimates when profiles were estimated.

Table E11) MMP Measurements in Latent Profile 1 vs 2 (Derivation Cohort)

	Latent Profile 1 (n=41)	Latent Profile 2 (n=85)	Significance
MMP-1 *	2450.1 (928.1-6995.0)	955.1 (631.1-1592.3)	**p=0.002**
MMP-2	751.5 (582.6-889.9)	409.4 (311.7-561.8)	**p<0.001**
MMP-3 *	13.5 (6.7-38.0)	2.6 (1.7-4.0)	**p<0.001**
MMP-7 *	1280.5 (747.5-4382.2)	405.1 (183.0-791.9)	**p<0.001**
MMP-8 *	56.1 (22.8-130.6)	19.7 (8.6-40.7)	**p<0.001**
MMP-9 *	64.0 (47.0-120.0)	140.5 (64.0-308.0)	**p=0.007**
MMP-9 (active)	152.6 (99.8-334.0)	269.0 (126.1-551.0)	**p=0.032**
TIMP-1 *	931.0 (702.0-1355.0)	197.5 (122.0-336.0)	**p<0.001**
TIMP-2 *	105.9 (83.3-124.6)	70.4 (54.9-83.5)	**p<0.001**
MMP-9/ TIMP-1	0.08 (0.06-0.15)	0.75 (0.41-1.66)	**p<0.001**
MMP-9 (active)/ **TIMP-1**	0.14 (0.10-0.31)	1.37 (0.68-2.98)	**p<0.001**

Legend: Biomarker distributions are described with median and interquartile ranges and compared across Profiles 1 vs. 2 with the non-parametric Wilcoxon rank sum test. * Indicates MMP biomarkers used to establish latent class membership.

Table E12) Multiple Linear Regression Associating Probability of Latent Profile 1 with MMP Pathway Measurements in the Derivation Cohort

Model	Adjusted R^2	Root MSE	k-1 Likelihood Ratio Test
TIMP-1	0.654	0.272	n/a
TIMP-1, MMP-3	0.689	0.258	**p<0.001**
TIMP-1, MMP-3, TIMP-2	0.737	0.234	**p<0.001**
TIMP-1, MMP-3, TIMP-2, MMP-7	0.767	0.220	**p<0.001**
TIMP-1, MMP-3, TIMP-2, MMP-7, MMP-9	0.816	0.196	**p<0.001**
TIMP-1, MMP-3, TIMP-2, MMP-7, MMP-9, MMP-1	0.817	0.195	p=0.217

Legend: Order of variables for subsequent addition into linear regression was selected by Least Angle Regression in order to maximize fit while minimizing C_p (Efron, Hastie, Johnstone and Tibshirani, The Annals of Statistics, 2004).

SUPPLEMENTAL DATA 3

Validation of Latent Classes

PEDIATRIC ARDS VALIDATION COHORT (n=109)

Table E13a) MMP Latent Profile Analysis in Pediatric ARDS Validation Cohort

Models	AIC	BIC	ABIC	k-1 LMR	Entropy	Profile Size
2 profiles	740.193	807.477	728.480	**p=0.003**	0.835	n=42,67
3 profiles	692.606	784.111	676.676	p=0.367	0.825	n=22,35,52
4 profiles	670.367	786.095	650.221	p=0.507	0.843	n=12,21,35,41

Legend: Akaike, Bayesian, and sample-size adjusted Bayesian Information Criteria (AIC, BIC, ABIC, respectively) are listed for 2, 3, and 4 profile models. Each model was compared to the model with 1 fewer latent profile (k-1) using the Lo-Mendell-Rubin (LMR) Adjusted Likelihood Ratio Test. Profile Size refers to sample size of each Latent Profile according to most likely assignment based on posterior probabilities. Although a 3 class model had a smaller BIC relative to the 2 profile model, it was not significantly different by LMR test (p=0.367) and had lower entropy; it was therefore was determined to be overfit and a 2 profile model was selected.

Table E13b) Average Latent Class Probabilities for Most Likely Latent Class Membership (Row) by Latent Class (Column) in the Validation Cohort

	Probability of Profile 1	Probability of Profile 2
Patients Most Likely to belong to Profile 1 (n=32)	97.0%	3.0%
Patients Most Likely to belong to Profile 2 (n=77)	5.9%	94.1%

Legend: Patients were assigned to latent profiles based on the maximum posterior probability for profile 1 vs. 2 (rows). The mean probability of being in profile 1 vs. profile 2 is listed for patients whose maximum posterior probability grouped them in profile 1 and again for patients whose maximum posterior probability grouped them in profile 2. These data show that misclassification in the 2-profile model was low.

Table E13c) Incidence and Mortality of Latent Profile 1 vs. 2 in the Validation Cohort

	Profile 1	Profile 2	Comparison
Number of Patients			
Maximum Posterior Probability	42 (38.5%)	67 (61.5%)	n/a
Estimated Profile Counts	41.6 (38.1%)	67.4 (61.9%)	n/a
Mortality			
Maximum Posterior Probability	13 (31.0%)	7 (10.5%)	**p=0.007**
Estimated Profile Counts	14.4 (34.5%)	5.4 (8.0%)	**p<0.001**

Legend: Profile counts were determined by maximum posterior probability or estimated according to each patient's probability of membership in each profile. Mortality counts were compared by Pearson chi-squared test when profiles were assigned by maximum posterior probability or by fitted regression estimates when profiles were estimated.

Table E14) MMP Measurements in Latent Profile 1 vs 2 (Validation Cohort)

	Latent Profile 1 (n=42)	Latent Profile 2 (n=67)	Significance
MMP-1 *	2455.7 (1032.4-4799.7)	844.0 (492.7-1965.0)	**p<0.001**
MMP-2	577.1 (484.2-795.8)	458.5 (348.0-711.2)	**p=0.014**
MMP-3 *	9.4 (3.8-20.0)	2.6 (1.5-5.0)	**p<0.001**
MMP-7 *	972.8 (366.4-1799.4)	179.1 (136.5-319.6)	**p<0.001**
MMP-8 *	56.4 (18.8-132.4)	21.5 (13.0-40.6)	**p=0.005**
MMP-9 *	80.5 (54.0-151.0)	145.0 (73.0-324.0)	**p=0.005**
MMP-9 (active)	133.4 (109.7-336.8)	235.1 (141.6-516.9)	p=0.072
TIMP-1 *	609.5 (396.0-1080.0)	189.0 (127.0-275.0)	**p<0.001**
TIMP-2 *	99.6 (80.9-118.0)	77.1 (60.1-89.8)	**p<0.001**
MMP-9/ TIMP-1	0.13 (0.07-0.18)	0.67 (0.35-1.54)	**p<0.001**
MMP-9 (active)/ **TIMP-1**	0.26 (0.13-0.54)	1.08 (0.70-2.48)	**p<0.001**

Legend: Biomarker distributions are described with median and interquartile ranges and compared across Profiles 1 vs. 2 with the non-parametric Wilcoxon rank sum test. * Indicates MMP biomarkers used to establish latent class membership.

Table E15) Latent Profile Assignments in the Validation Cohort

	Latent Profile 1 by LPA	Latent Profile 2 by LPA
Profile 1 by Regression from Derivation Cohort	28 (35.9%)	1 (1.3%)
Profile 2 by Regression from Derivation Cohort	2 (2.6%)	47 (60.3%)

Legend: n=78 of 109 validation cohort patients included (n=31 excluded due to inability to apply regression given missing MMP pathway measurements).

Table E16) MMP Profiles are Associated with Elevated Inflammation, Endothelial Injury, and Impaired Oxygenation

	Derivation Group			Validation Group		
	Latent Profile 1	**Latent Profile 2**	**Signif.**	**Latent Profile 1**	**Latent Profile 2**	**Signif.**
Inflammation						
IL-1RA	777.5 (470-6270)	488 (190-823.5)	**p=0.002**	793 (17-2550)	384 (0-711)	**p=0.002**
IL-6	174 (50-1465)	53 (21-137)	**p=0.004**	228.5 (75-1610)	39 (11-141)	**p<0.001**
IL-8	300.5 (160.5-918.5)	87 (40.5-176)	**p<0.001**	244 (158-584)	63 (34-114)	**p<0.001**
IL-10	74 (38-328)	22 (8.6-48)	**p<0.001**	51.5 (35-88)	16 (9.6-49)	**p<0.001**
IL-18	1180 (643.5-1925)	345 (192.5-750)	**p<0.001**	599 (334-1960)	352 (189-729)	**p<0.001**
MIP-1α	60 (36-96)	29 (0-35)	**p<0.001**	38.5 (0-60)	0 (0-0)	**p<0.001**
MIP-1β	648 (436.5-1130)	353.5 (225-454)	**p<0.001**	510.5 (374-870)	315 (206-442)	**p<0.001**
TNF-α	4.1 (2.7-9.5)	1.7 (1.3-2.5)	**p<0.001**	4.2 (2.6-7.2)	1.6 (1.2-2.5)	**p<0.001**
TNF-R2	94 (41.5-148.5)	18 (12.5-33)	**p<0.001**	37.5 (20-78)	15 (10-21)	**p<0.001**
Endothelial Injury						
Ang-2	24.6 (10.3-43.0)	5.7 (3.2-9.2)	**p<0.001**	12.8 (7.7-24.2)	4.8 (2.8-9.4)	**p<0.001**
vWF	265 (199-334)	233 (136-283)	**p=0.036**	243.5 (204-344)	200 (162-286)	**p=0.005**
sTM	149.0 (86.1-247.0)	69.2 (46.2-99.8)	**p<0.001**	115.3 (77.0-167.7)	83.4 (47.6-117.2)	**p=0.001**
Oxygenation						
PaO2/FiO2 Ratio	108.6 (79-240)	177.9 (92.1-228.3)	p=0.105	126 (82.5-172)	123.5 (75-205.7)	p=0.989
Oxygenation Index	12.4 (6.8-22.1)	7.9 (6.0-15.6)	p=0.086	12.2 (7.4-22.7)	9.3 (5.4-17.1)	**p=0.036**

Legend: Biomarker distributions are described with median and interquartile ranges and compared across Profiles 1 vs. 2 with the non-parametric Wilcoxon rank sum test.

Table E17) Receiver Operating Characteristics for Clinical Outcomes

	Mortality		Mortality or Severe Morbidity	
P/F	0.65	0.56-0.74	0.66	0.58-0.73
OI	0.67	0.58-0.76	0.69	0.62-0.77
MMP Profile	0.63	0.55-0.71	0.69	0.62-0.75
P/F + MMP Profile	0.72	0.62-0.81	0.75	0.68-0.82
OI + MMP Profile	0.72	0.63-0.82	0.77	0.70-0.84

Legend: Areas under the receiver operating characteristics (AUROC) are shown with 95% confidence intervals. P/F n=218, OI n=198, MMP n=235, P/F + MMP n=218, OI + MMP n=198. Comparisons of models (ie: P/F vs. P/F + MMP Profile) are listed in the primary text and used only the subset of observations present in both models.