

Supplemental Content for “Serial Measurements of Protein Biomarkers in Sepsis-Induced Acute Respiratory Distress Syndrome”

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Supplemental Table S1. The Berlin definition for acute respiratory distress syndrome and special considerations

Criteria	Definition
Timing	Within 1 week of a known clinical insult, which was sepsis or septic shock in this study
Chest imaging	Bilateral opacities, not fully explained by effusions, lung collapse, or nodules
Origin of edema	Respiratory failure not fully explained by cardiac failure or fluid overload
Oxygenation Mild Moderate Severe	200 mm Hg < PaO ₂ /FiO ₂ ≤ 300 mm Hg with PEEP or CPAP ≥ 5cmH ₂ O 100 mm Hg < PaO ₂ /FiO ₂ ≤ 200 mm Hg with PEEP ≥ 5cmH ₂ O PaO ₂ /FiO ₂ ≤ 100 mm Hg with PEEP ≥ 5cmH ₂ O
Special considerations	<ul style="list-style-type: none"> • Qualifying chest imaging and qualifying P/F ratio must be within 24 hours of each other. • The chest imaging abnormalities and P/F ratio < 300 must persist for at least two days, whenever data is available. • For patients without P/F ratio available, S/F ratio was used as needed. • For participants receiving oxygen support with heated and humidified HFNC, the fraction of inspired oxygen (FiO₂) setting on the HFNC was used to calculate the P/F or S/F ratios, but they were not considered to fulfill the Berlin definition of ARDS unless they subsequently received either non-invasive ventilation (NIV) or invasive mechanical ventilation (IMV). • For those subsequently requiring NIPPV or IMV, a new P/F or S/F ratio after initiation of positive-pressure ventilation was used as the qualifying measure for ARDS.

Abbreviations: PaO₂/FiO₂ or P/F = ratio of partial pressure arterial oxygen to fraction of inspired oxygen, PEEP = positive end-expiratory pressure, CPAP = continuous positive airway pressure, S/F = ratio of peripheral oxygen saturation to fraction of inspired oxygen, HFNC = high-flow nasal cannula, ARDS = acute respiratory distress syndrome.

Supplemental Table S2. Comparison of biomarker levels by ARDS diagnosis

Biomarker	ARDS (n=21, 18.9%)	Non-ARDS (n=90, 81.1%)	p-value*
sRAGE levels			
Day 1 (log[ng/mL])	1.042 ± 1.002	0.629 ± 0.980	0.09
Day 2 (log[ng/mL])	0.853 ± 0.994	0.535 ± 1.003	0.22
Day 3 (log[ng/mL])	0.608 ± 1.027	0.480 ± 1.082	0.67
Δ day 1 to 2 (ng/mL)	-0.120 (-1.240 – 0.102)	-0.076 (-0.367 – 0.284)	0.23
Δ day 1 to 3 (ng/mL)	-0.514 (-1.552 – -0.075)	-0.153 (-0.800 – 0.359)	0.06
Ang-2 levels			
Day 1 (log[ng/mL])	1.825 ± 0.616	1.893 ± 0.722	0.69
Day 2 (log[ng/mL])	1.836 ± 0.683	1.745 ± 0.748	0.64
Day 3 (log[ng/mL])	1.720 ± 0.631	1.672 ± 0.706	0.80
Δ day 1 to 2 (ng/mL)	0.380 (-1.117 – 1.898)	-0.511 (-1.979 – 0.190)	0.049
Δ day 1 to 3 (ng/mL)	-0.440 (-1.324 – 0.558)	-1.005 (-2.965 – 0.016)	0.19
SP-D levels			
Day 1 (log[ng/mL])	1.921 ± 1.244	1.361 ± 1.211	0.06
Day 2 (log[ng/mL])	2.009 ± 1.258	1.456 ± 1.286	0.10
Day 3 (log[ng/mL])	1.900 ± 1.089	1.606 ± 1.325	0.41
Δ day 1 to 2 (ng/mL)	0.856 (-0.250 – 2.468)	0.214 (-1.025 – 2.363)	0.32
Δ day 1 to 3 (ng/mL)	1.084 (-1.923 – 3.948)	0.556 (-0.938 – 3.866)	0.68

Abbreviations: ARDS = acute respiratory distress syndrome, sRAGE = soluble receptor for advanced glycation end-products, Ang-2 = angiotensin-2, SP-D = surfactant protein-D.

Absolute biomarker levels on each day were log-transformed to approximate a normal distribution, and presented as mean ± standard deviation. Changes of the biomarker levels over time (denoted by the symbol Δ) are not log-transformed, and presented as median (interquartile range).

*Two-sample t-test was used for calculating the p-values when comparing the means of the absolute biomarker levels, and Wilcoxon rank-sum test was used for calculating the p-values when comparing the medians of the changes of the biomarker levels over time.

Supplemental Table S3. Comparison of biomarker levels by mortality status

Biomarker	Non-survivors (n=45, 40.5%)	Survivors (n=66, 59.5%)	p-value*
sRAGE levels			
Day 1 (log[ng/mL])	0.999 ± 0.964	0.508 ± 0.970	0.01
Day 2 (log[ng/mL])	0.848 ± 0.876	0.428 ± 1.052	0.04
Day 3 (log[ng/mL])	0.736 ± 0.898	0.367 ± 1.141	0.13
Δ day 1 to 2 (ng/mL)	-0.005 (-0.642 – 0.595)	-0.112 (-0.412 – 0.098)	0.27
Δ day 1 to 3 (ng/mL)	-0.096 (-1.281 – 0.389)	-0.234 (-0.792 – 0.207)	0.90
Ang-2 levels			
Day 1 (log[ng/mL])	2.065 ± 0.673	1.754 ± 0.697	0.02
Day 2 (log[ng/mL])	2.044 ± 0.652	1.581 ± 0.732	0.002
Day 3 (log[ng/mL])	1.973 ± 0.652	1.508 ± 0.656	0.002
Δ day 1 to 2 (ng/mL)	0.050 (-2.051 – 1.898)	-0.563 (-1.535 – 0.062)	0.12
Δ day 1 to 3 (ng/mL)	-0.829 (-3.856 – 0.381)	-0.744 (-2.424 – -0.020)	0.91
SP-D levels			
Day 1 (log[ng/mL])	1.792 ± 1.287	1.245 ± 1.150	0.02
Day 2 (log[ng/mL])	1.772 ± 1.321	1.418 ± 1.265	0.18
Day 3 (log[ng/mL])	1.975 ± 1.182	1.477 ± 1.314	0.08
Δ day 1 to 2 (ng/mL)	-0.093 (-1.913 – 2.468)	0.303 (-0.542 – 2.370)	0.26
Δ day 1 to 3 (ng/mL)	0.657 (-3.188 – 6.681)	0.676 (-0.661 – 3.231)	0.37

Abbreviations: sRAGE = soluble receptor for advanced glycation end-products, Ang-2 = angiotensin-2, SP-D = surfactant protein-D.

Absolute biomarker levels on each day were log-transformed to approximate a normal distribution, and presented as mean ± standard deviation. Changes of the biomarker levels over time (denoted by the symbol Δ) are not log-transformed, and presented as median (interquartile range).

*Two-sample t-test was used for calculating the p-values when comparing the means of the absolute biomarker levels, and Wilcoxon rank-sum test was used for calculating the p-values when comparing the medians of the changes of the biomarker levels over time.

Supplemental Table S4. Comparison of biomarker levels by ARDS diagnosis in subgroup of patients with COVID-19

Biomarker	ARDS (n=8, 42.1%)	Non-ARDS (n=11, 57.9%)	p-value*
sRAGE levels			
Day 1 (log[ng/mL])	1.389 ± 0.637	1.110 ± 0.767	0.41
Day 2 (log[ng/mL])	1.168 ± 0.735	0.951 ± 0.587	0.49
Day 3 (log[ng/mL])	1.129 ± 0.702	0.838 ± 0.446	0.32
Δ day 1 to 2 (ng/mL)	-0.120 (-1.240 – 0.102)	-0.076 (-0.367 – 0.284)	0.51
Δ day 1 to 3 (ng/mL)	-0.514(-1.552 – -0.075)	-0.153 (-0.799 – 0.359)	0.67
Ang-2 levels			
Day 1 (log[ng/mL])	1.589 ± 0.470	1.811 ± 0.738	0.46
Day 2 (log[ng/mL])	1.493 ± 0.481	1.962 ± 0.724	0.14
Day 3 (log[ng/mL])	1.521 ± 0.652	1.873 ± 0.776	0.33
Δ day 1 to 2 (ng/mL)	0.380 (-1.117 – 1.898)	-0.511 (-1.979 – 0.190)	0.16
Δ day 1 to 3 (ng/mL)	-0.440 (-1.324 – 0.558)	-1.005 (-2.965 – 0.016)	0.74
SP-D levels			
Day 1 (log[ng/mL])	1.882 ± 1.241	1.551 ± 1.598	0.63
Day 2 (log[ng/mL])	1.931 ± 1.284	2.034 ± 1.451	0.88
Day 3 (log[ng/mL])	1.536 ± 1.079	2.239 ± 1.114	0.21
Δ day 1 to 2 (ng/mL)	0.856 (-0.250 – 2.468)	0.214 (-1.025 – 2.363)	0.57
Δ day 1 to 3 (ng/mL)	1.084 (-1.923 – 3.948)	0.556 (-0.938 – 3.866)	0.43

Abbreviations: ARDS = acute respiratory distress syndrome, COVID-19 = coronavirus disease-2019, sRAGE = soluble receptor for advanced glycation end-products, Ang-2 = angiotensin-2, SP-D = surfactant protein-D.

Absolute biomarker levels on each day were log-transformed to approximate a normal distribution, and presented as mean ± standard deviation. Changes of the biomarker levels over time (denoted by the symbol Δ) are not log-transformed, and presented as median (interquartile range).

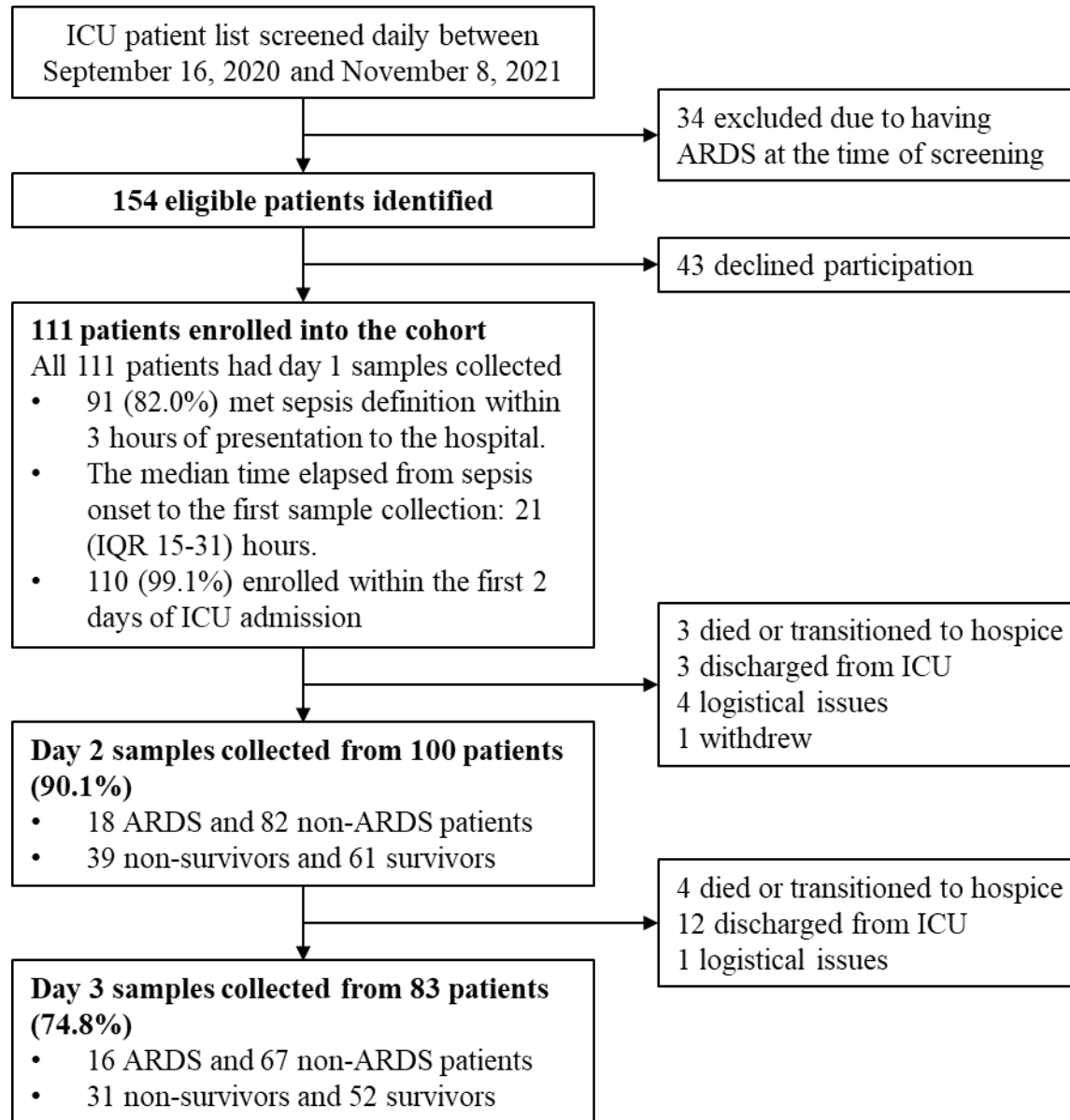
*Two-sample t-test was used for calculating the p-values when comparing the means of the absolute biomarker levels, and Wilcoxon rank-sum test was used for calculating the p-values when comparing the medians of the changes of the biomarker levels over time.

Supplemental Table S5. Coefficients of variations for ELISA analyses

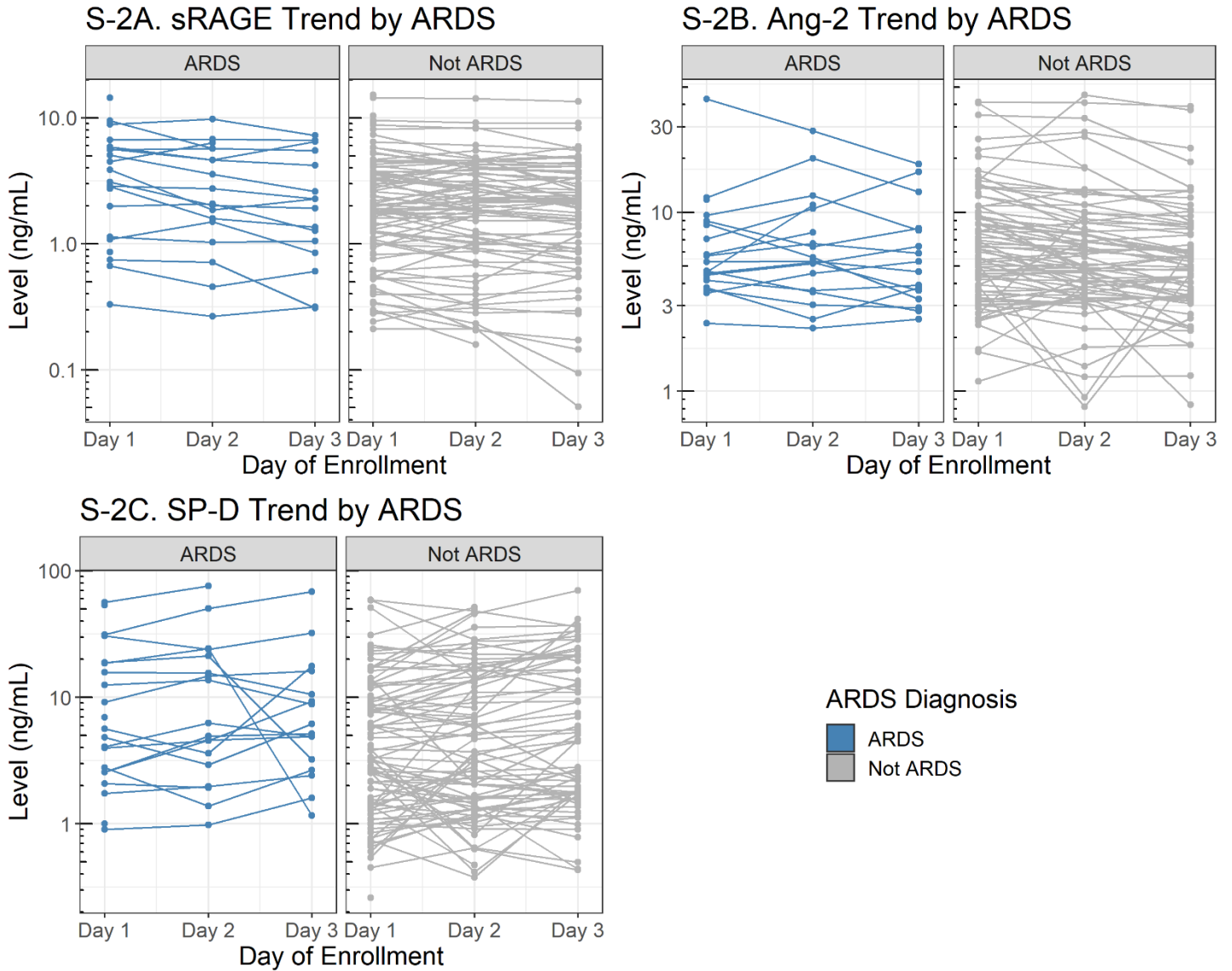
Biomarker	Inter-assay coefficient of variation
sRAGE	4.98%
Ang-2	4.94%
SP-D	7.40%

Abbreviations: ELISA = enzyme-linked immunosorbent assay, sRAGE = soluble receptor for advanced glycation end-products, Ang-2 = angiotensin-2, SP-D = surfactant protein-D. Coefficients of variation <15% is generally considered acceptable.

Supplemental Figure S1. Flowchart of study enrollment and sample collection

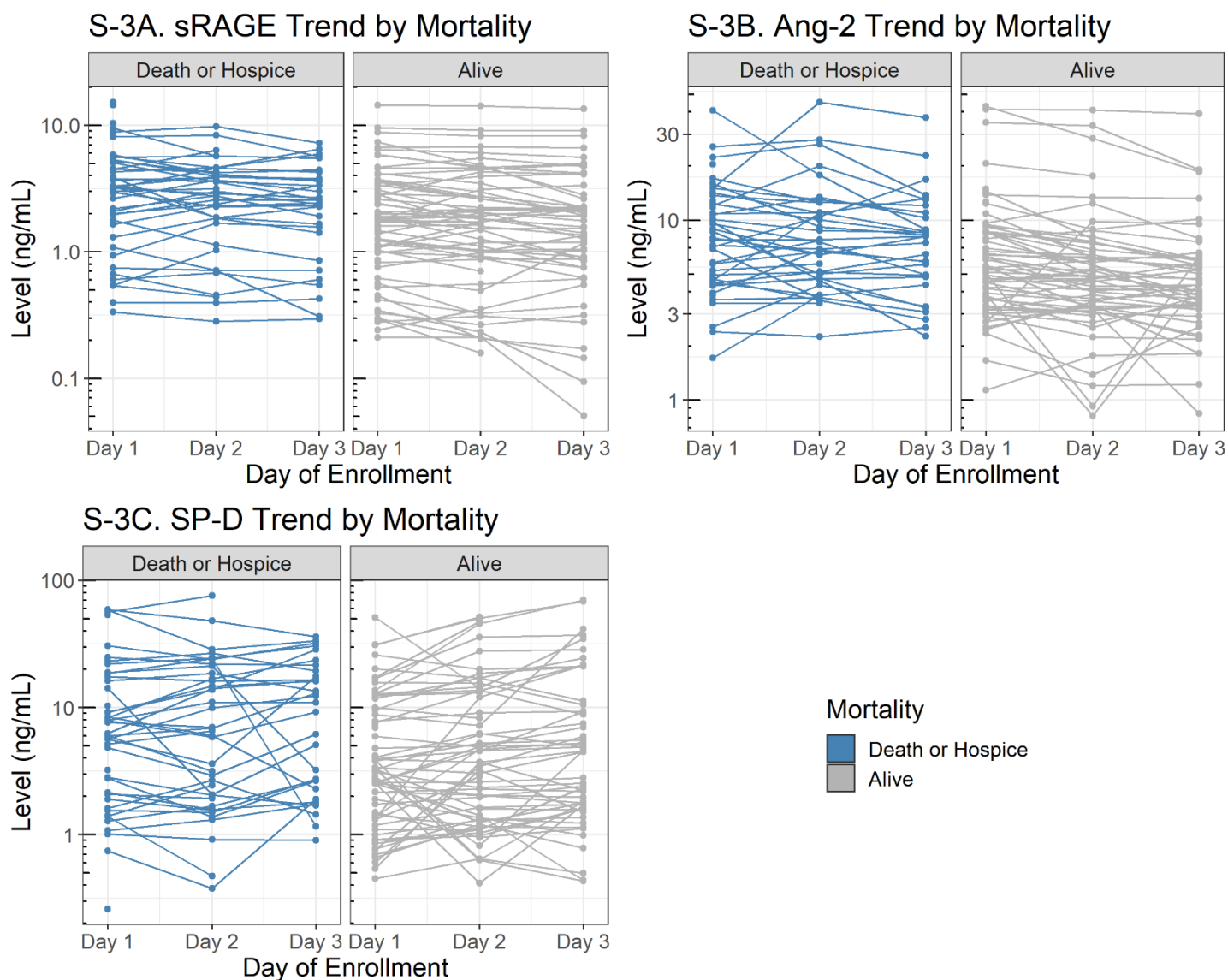


Supplemental Figure S2. Biomarker trends over time by ARDS diagnosis



Abbreviations: ARDS = acute respiratory distress syndrome, sRAGE = soluble receptor for advanced glycation end-products, Ang-2 = angiotensin-2, SP-D = surfactant protein D. Each panel shows the trends of the three biomarker levels over time in each patient. Number of patients for each day was: 111 on day 1 (21 ARDS vs. 90 non-ARDS), 100 on day 2 (18 ARDS vs. 82 non-ARDS), and 83 on day 3 (16 ARDS vs. 67 non-ARDS).

Supplemental Figure S3. Biomarker trends over time by mortality status



Abbreviations: sRAGE = soluble receptor for advanced glycation end-products, Ang-2 = angiotensin-2, SP-D = surfactant protein D. Each panel shows the trends of the three biomarker levels over time in each patient. Number of patients for each day was: 111 on day 1 (45 death/hospice vs. 66 alive), 100 on day 2 (39 death/hospice vs. 61 alive) and 83 on day 3 (31 death/hospice vs. 52 alive).