"Incorporating Inflammation into Mortality Risk in Pediatric Acute Respiratory Distress Syndrome"

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Supplemental Text

Supplemental Methods, Confounding Variables:

We defined infectious trigger of ARDS as pneumonia, sepsis, or other documented infection considered an inciting cause of ARDS; trauma, aspiration, TRALI, and other etiologies of lung injury were considered non-infectious triggers of ARDS.

Supplemental Methods: Measurements:

The Luminex multiplex immunoassay (Myriad RBM, Austin, TX, USA) measured IL-6, IL-8, IL-18, MIP-1β, IL-1RA, IL-10, and TNF-R2 with values above the lower limit of quantitation (LLOQ) in greater than 75% of patients. When these biomarkers were detected but below the lower limit of quantitation (LLOQ) of the assay, values were assumed to be zero for all analyses. The Luminex Human InflammationMAP® 1.0 multiplex immunoassay also measured IFN-γ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-7, IL-12, IL-15, IL-17, IL-23, MIP-1α, and TNF-α but had values below the lower limit of quantitation (LLOQ) in greater than 75% of patients, and therefore was assumed to be insufficiently sensitive to use these data. Intraassay variability was tested by

performing the assay in duplicate on a subset of 25 samples on the same day by the same user and only measurements with ≤15% intraassay variability were retained.

Supplemental Methods, Statistics:

Categorical variables were compared with the Fisher Exact test. All continuous variables were non-normally distributed and therefore were reported as median values with interquartile ranges (IQR) and compared using the Wilcoxon rank sum test or the Spearman correlation coefficient followed by multivariable linear regression to adjust for covariates as appropriate (1, 2). Mortality odds ratios were computed from multiple logistic regression with $\log_{10}$-transformation of non-normally distributed continuous variables, with two-tailed p-values based on robust standard error estimates with a nominal significance level of α=0.05. Model calibration was assessed by computing sensitivity and specificity for patients in the top quartile of predicted risk; goodness of fit was assessed with the Hosmer-Lemeshow goodness of fit Chi squared test (3). To test for the mortality discrimination of our measured biomarkers, we generated the receiver operating characteristic (ROC) for each biomarker and calculated the area under the curve (AUC) with 95% CI. AUROC were compared using a nonparametric covariance matrix with significance estimated by a 2-tailed Chi squared statistic (4). We also compared multivariable models using likelihood ratios (5). All analyses were performed on STATA software 13.1 (StataCorp, College Station, TX, USA).

Supplemental Table 1) Characteristics of Enrolled Patients with vs without Biomarker Measurements

	All patients (n=284)	**Biomarkers Measured** (n=194)	**Biomarkers not Measured** (n=90)	**Significance**
Age (median years, IQR)	4.8 (1.0-11.5)	4.9 (0.9-11.5)	4.6 (1.0-11.3)	p=0.769
Male (n, %)	158 (55.6)	107 (55.2)	51 (56.7)	p=0.898
Race (n, %)				**p=0.015**
White	179 (63.0)	135 (69.6)	44 (48.9)	
Unknown	35 (12.3)	22 (11.3)	13 (14.4)	
Multiple	24 (8.5)	14 (7.2)	12 (13.3)	
Black	25 (8.8)	10 (5.2)	11 (12.2)	
Asian/PI	18 (6.3)	12 (6.2)	8 (8.9)	
American Indian	3 (1.1)	1 (0.5)	2 (2.2)	
Lung Injury Etiology (n, %)				p=0.246
Pneumonia	168 (59.6)	109 (56.2)	59 (67.1)	
Sepsis	57 (20.2)	42 (21.7)	15 (17.1)	
Other	25 (8.9)	16 (8.3)	9 (10.2)	
Trauma	16 (5.7)	12 (6.2)	4 (4.6)	
Aspiration	11 (3.9)	10 (5.2)	1 (1.1)	
TRALI	5 (1.8)	5 (2.6)	0 (0)	
Infection (n, %)	225 (79.8)	151 (77.8)	74 (84.1)	p=0.264
HCT (n, %)	24 (8.5)	21 (10.8)	3 (3.3)	**p=0.039**
Days in PICU Prior to ARDS Onset (median, IQR)	1 (0-2)	1 (0-2)	1 (0-2)	p=0.623
Day 1 Oxygenation Metric (n, %)				**p=0.017**
Arterial Line (PaO2)	235 (82.8)	168 (86.6)	67 (74.4)	
Pulse Oximeter (SpO2)	49 (17.2)	26 (13.4)	23 (25.6)	
Day 1 Respiratory Support (n, %)				p=0.850
Noninvasive (CPAP/BiPAP)	14 (5.0)	9 (4.6)	5 (5.6)	
Invasive (Conventional)	255 (90.1)	176 (90.7)	79 (88.8)	
Invasive (HFOV)	14 (5.0)	9 (4.6)	5 (5.6)	
Day 1 Illness Severity (median, IQR)				
PaO2/FiO2 Ratio (P/F)	132 (89.5-215)	136.5 (90.1-218)	128 (82.6-208)	p=0.701
Oxygenation Index (OI)	10.0 (5.3-18.3)	9.4 (5.3-18.3)	11.2 (5.1-18.0)	p=0.752
PRISM-3*	13 (8-20)	13 (8-21)	12 (8-16)	p=0.298

Legend: Associations tested with Fisher exact test for categorical variables and Wilcoxon rank sum for non-normally distributed continuous variables. For all patients, P/F ratio n=268, OI n=263, PRISM-3 n=179. *PRISM-3 was calculated for n=254 patients but overlapped with ARDS day 0 or 1 in n=179 patients.

Supplemental Table 2) Cytokine Associations with Mortality in Pediatric ARDS.

		AUROC	Different than AUROC of OI?
Pro-	**IL-6**	0.63 (0.53-0.73)	p=0.682
	IL-8	0.68 (0.60-0.77)	p=0.593
	IL-18	0.61 (0.50-0.72)	p=0.422
	MIP-1β	0.59 (0.49-0.68)	p=0.362
	TNF-α	0.53 (0.41-0.64)	p=0.183
Anti-	**IL-1RA**	0.55 (0.44-0.65)	p=0.159
	IL-10	0.61 (0.50-0.71)	p=0.540
	TNF-R2	0.60 (0.51-0.70)	p=0.526

Legend: AUROC were compared using a nonparametric covariance matrix with significance estimated by a 2-tailed Chi squared statistic. Mortality was defined as all-cause hospital mortality.

Supplemental Table 3) Cytokine Associations with HCT

		HCT (n=21)	**No HCT** (n=173)	**Significance**	**Logistic Regression (OR)**
Pro-	**IL-6**	74.5 (18.5-302)	73.5 (25-257)	p=0.858	0.9 (0.5-1.5, p=0.648)
	IL-8	**188 (111-285)**	**97 (47-229)**	**p=0.010**	**2.2 (1.1-4.4, p=0.028)**
	IL-18	**1440 (990-2320)**	**445 (233-925)**	**p<0.001**	**9.7 (3.0-30.9, p<0.001)***
	MIP-1β	436 (309-512)	376 (255-630)	p=0.395	1.8 (0.6-4.9, p=0.263)
	TNF-α	2.9 (1.3-6.2)	2.3 (1.3-3.6)	p=0.296	2.0 (0.7-5.4, p=0.195)
Anti-	**IL-1RA**	318 (0-902)	544 (262-950)	p=0.275	0.7 (0.5-1.1, p=0.058)
	IL-10	**58 (30-97)**	**31 (11-65)**	**p=0.017**	**2.0 (1.1-3.7, p=0.020)***
	TNF-R2	**46 (22-99)**	**20 (13-41)**	**p=0.004**	**5.4 (1.9-15.9, p=0.002)***

Legend: Cytokine levels expressed as median pg/mL with IQR. Associations were tested using Wilcoxon Rank Sum. Logistic regression performed for outcome of HCT, predictor of $\log_{10}$-transformed cytokine level, and adjustment for age, sex, and race. Odds ratio and p-value are reported. *HCT remained positively associated with IL-18, IL-10, and TNF-R2 upon further adjustment for infectious ARDS trigger. (p<0.001, p=0.044, and p=0.008, respectively).

Supplemental Table 4) Adjusted Odds of Mortality

		Adjusted Mortality Odds		
		adjusted for age, sex, race:	*adjusted for age, sex, race, HCT:*	*adjusted for age, sex, race, HCT, OI:*
Pro-	**IL-6**	**1.8 (1.2-2.7, p=0.008)**	**2.0 (1.3-3.2, p=0.003)**	**1.8 (1.1-2.9, p=0.018)**
	IL-8	**2.9 (1.5-5.4, p=0.001)**	**2.6 (1.3-5.0, p=0.006)**	**2.3 (1.1-4.6, p=0.024)**
	IL-18	2.1 (0.9-4.7, p=0.063)	1.2 (0.5-2.9, p=0.752)	0.9 (0.3-2.4, p=0.805)
	MIP-1β	2.2 (0.9-5.3, p=0.073)	2.0 (0.8-5.2, p=0.145)	2.0 (0.8-5.3, p=0.150)
	TNF-α	1.4 (0.6-3.3, p=0.472)	1.1 (0.4-2.8, p=0.850)	1.1 (0.4-3.0, p=0.827)
Anti-	**IL-1RA**	1.0 (0.8-1.3, p=0.997)	1.1 (0.8-1.5, p=0.457)	1.1 (0.8-1.5, p=0.560)
	IL-10	1.6 (0.9-2.7, p=0.055)	1.4 (0.8-2.3, p=0.278)	1.4 (0.8-2.4, p=0.276)
	TNF-R2	**2.5 (1.1-6.0, p=0.037)**	1.6 (0.6-4.2, p=0.354)	1.6 (0.6-4.3, p=0.396)

Legend: Association between pro- and anti-inflammatory cytokines and mortality. OR calculated through multivariable logistic regression and expressed per $\log_{10}$ change in cytokine.

Supplemental Table 5) Cytokine Association with PRISM-3, P/F Ratio, and Oxygenation Index

		P/F Ratio	Oxygenation Index	PRISM-3
Pro-	**IL-6**			
	Spearman	**ρ=-0.258 (p<0.001)**	**ρ=0.266 (p<0.001)**	**ρ=0.260 (p=0.004)**
	Linear Regression	**β=-28.03 (p=0.001)**	**β=3.34 (p=0.006)**	**β=2.12 (p=0.002)**
	IL-8			
	Spearman	**ρ=-0.257 (p<0.001)**	**ρ=0.317 (p<0.001)**	**ρ=0.453 (p<0.001)**
	Linear Regression	**β=-34.84 (p=0.006)**	**β=4.54 (p=0.014)**	**β=5.37 (p<0.001)**
	IL-18			
	Spearman	**ρ=-0.209 (p=0.004)**	**ρ=0.275 (p<0.001)**	**ρ=0.374 (p<0.001)**
	Linear Regression	**β=-48.50 (p=0.003)**	**β=7.97 (p=0.001)**	**β=5.81 (p<0.001)**
	MIP-1β			
	Spearman	ρ=-0.124 (p=0.085)	**ρ=0.168 (p=0.020)**	**ρ=0.280 (p=0.002)**
	Linear Regression	β=-23.47 (p=0.215)	β=4.18 (p=0.123)	**β=4.59 (p=0.001)**
	TNF-α			
	Spearman	ρ=-0.068 (p=0.408)	**ρ=0.094 (p=0.256)**	**ρ=0.430 (p<0.001)**
	Linear Regression	β=-13.01 (p=0.474)	β=1.85 (p=0.500)	**β=5.44 (p<0.001)**
Anti-	**IL-1RA**			
	Spearman	**ρ=-0.222 (p=0.002)**	**ρ=0.284 (p<0.001)**	**ρ=0.349 (p<0.001)**
	Linear Regression	**β=-16.48 (p=0.002)**	**β=1.89 (p=0.013)**	**β=1.58 (p<0.001)**
	IL-10			
	Spearman	**ρ=-0.205 (p=0.004)**	**ρ=0.251 (p<0.001)**	**ρ=0.376 (p<0.001)**
	Linear Regression	**β=-26.05 (p=0.012)**	β=2.19 (p=0.141)	**β=3.82 (p<0.001)**
	TNF-R2			
	Spearman	ρ=-0.118 (p=0.102)	**ρ=0.233 (p=0.001)**	**ρ=0.574 (p<0.001)**
	Linear Regression	**β=-36.01 (p=0.047)**	**β=5.80 (p=0.026)**	**β=9.51 (p<0.001)**

Legend: Associations were tested using Spearman Correlation with rho and p-value reported. Linear regression performed for outcome of P/F ratio, OI, or PRISM-3, predictor of $\log_{10}$-transformed cytokine level, and adjusted for age, sex, and race. Regression coefficient and p-value are reported.

Supplemental Table 6) Cytokine Associations with Survivor ICU LOS and PELOD

		Survivor ICU LOS	Survivor PELOD
Pro-	**IL-6**		
	Spearman	ρ=0.150 (p=0.063)	**ρ=0.203 (p=0.012)**
	Linear Regression	β=-0.97 (p=0.752)	**β=2.84 (p=0.013)**
	IL-8		
	Spearman	ρ=0.079 (p=0.330)	**ρ=0.382 (p<0.001)**
	Linear Regression	β=-6.40 (p=0.168)	**β=7.63 (p<0.001)**
	IL-18		
	Spearman	ρ=0.040 (p=0.620)	**ρ=0.322 (p<0.001)**
	Linear Regression	β=-5.48 (p=0.353)	**β=9.01 (p<0.001)**
	MIP-1β		
	Spearman	**ρ=0.245 (p=0.002)**	**ρ=0.188 (p=0.019)**
	Linear Regression	β=1.78 (p=0.794)	**β=6.73 (p=0.008)**
	TNF-α		
	Spearman	ρ=0.096 (p=0.303)	**ρ=0.341 (p<0.001)**
	Linear Regression	β=-0.18 (p=0.955)	**β=8.96 (p<0.001)**
Anti-	**IL-1RA**		
	Spearman	ρ=0.049 (p=0.545)	**ρ=0.208 (p=0.009)**
	Linear Regression	β=-1.06 (p=0.575)	β=1.08 (p=0.125)
	IL-10		
	Spearman	ρ=0.135 (p=0.092)	**ρ=0.265 (p<0.001)**
	Linear Regression	β=-1.40 (p=0.704)	**β=4.72 (p<0.001)**
	TNF-R2		
	Spearman	**ρ=0.103 (p=0.201)**	**ρ=0.430 (p<0.001)**
	Linear Regression	β=-8.98 (p=0.166)	**β=13.16 (p<0.001)**

Legend: Associations were tested using Spearman Correlation with rho and p-value reported. Linear regression performed for outcome of Survivor ICU LOS or Survivor PELOD, predictor of $\log_{10}$-transformed cytokine level, and adjusted for age, sex, and race; regression coefficient, and p-value are reported.

Supplemental Table 7) Correlation Matrix for Cytokines and Biomarkers of Endothelial Injury

	IL-6	IL-8	IL-18	MIP-1β	TNF-α	IL-1RA	IL-10	TNF-R2	Ang-2	vWF	sTM
IL-6	**1**										
IL-8	**0.648 (p<0.001)**	**1**									
IL-18	0.251 (p=0.248)	**0.510 (p<0.001)**	**1**								
MIP-1β	**0.512 (p<0.001)**	**0.642 (p<0.001)**	**0.463 (p<0.001)**	**1**							
TNF-α	**0.405 (p<0.001)**	**0.612 (p<0.001)**	**0.523 (p<0.001)**	**0.661 (p<0.001)**	**1**						
IL-1RA	**0.564 (p<0.001)**	**0.595 (p<0.001)**	**0.346 (p=0.004)**	**0.545 (p<0.001)**	**0.566 (p<0.001)**	**1**					
IL-10	**0.513 (p<0.001)**	**0.643 (p<0.001)**	**0.505 (p<0.001)**	**0.607 (p<0.001)**	**0.611 (p<0.001)**	**0.621 (p<0.001)**	**1**				
TNF-R2	**0.474 (p<0.001)**	**0.701 (p<0.001)**	**0.699 (p<0.001)**	**0.614 (p<0.001)**	**0.763 (p<0.001)**	**0.607 (p<0.001)**	**0.712 (p<0.001)**	**1**			
Ang-2	**0.463 (p<0.001)**	**0.443 (p<0.001)**	0.253 (p=0.230)	0.185 (p=1.000)	**0.373 (p<0.001)**	0.225 (p=0.604)	**0.328 (p=0.009)**	**0.402 (p<0.001)**	1		
vWF	0.230 (p=0.505)	0.178 (p=1.000)	0.190 (p=1.000)	0.058 (p=1.000)	0.082 (p=1.000)	0.038 (p=1.000)	0.210 (p=0.970)	0.230 (p=0.506)	0.268 (p=0.129)	1	
sTM	0.255 (p=0.208)	**0.421 (p<0.001)**	0.157 (p=1.000)	0.221 (p=0.684)	0.249 (p=0.262)	**0.379 (p<0.001)**	**0.389 (p<0.001)**	**0.395 (p<0.001)**	0.271 (p=0.113)	0.115 (p=1.000)	**1**

Legend: Associations tested using Spearman's Correlation Coefficient All pairwise comparisons have significant Spearman's correlation with Bonferroni adjusted p-values <0.05 to account for multiple comparisons.

Supplemental Table 8): Calibration of Multivariable Logistic Model for Mortality or Severe Morbidity in Pediatric ARDS

Probability of Death or Severe Morbidity (%)	Expected Deaths	Observed Deaths
0 ≤ n < 18.3 (n=48)	6.5	6
18.3 ≤ n < 32.4 (n=48)	12	13
32.4 ≤ n < 53.6 (n=48)	20.9	21
53.6 ≤ n (n=47)	35.6	35
Total (n=191)	75	75

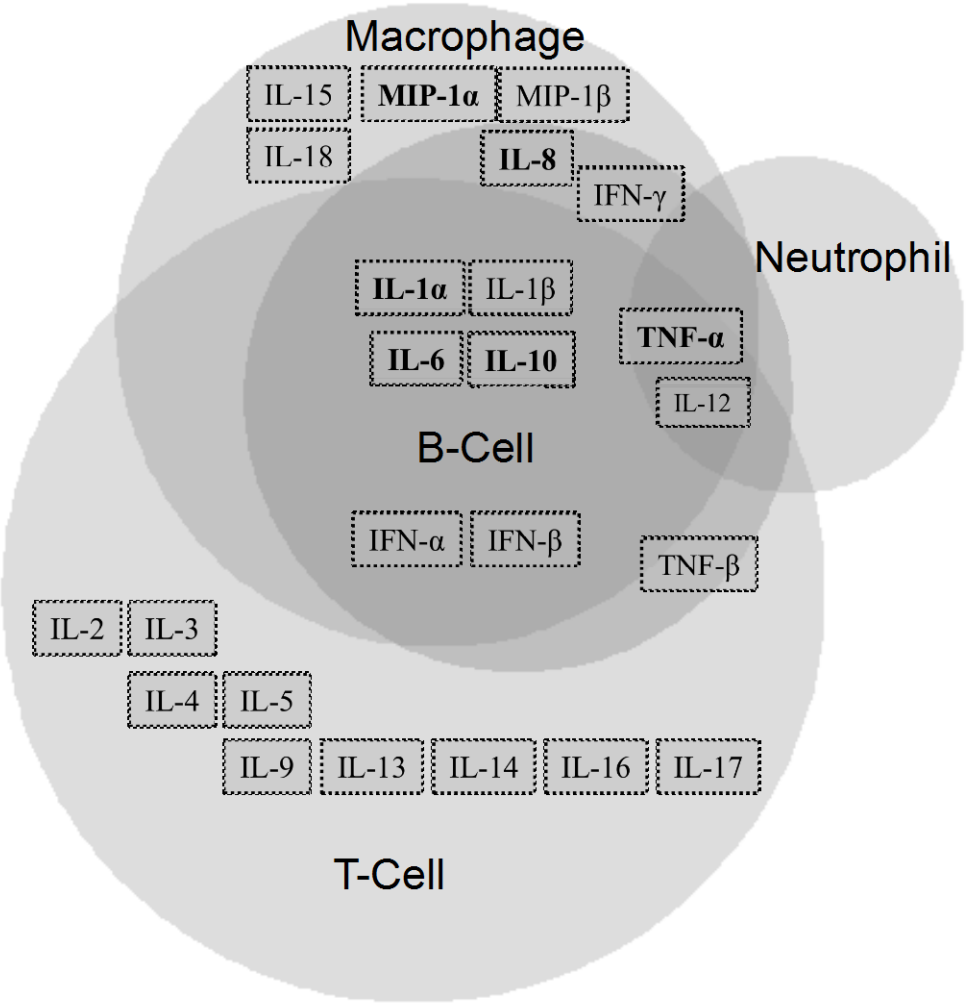
Legend: Hosmer-Lemeshow goodness-of-fit χ^2 =0.21, 4 groups, p=0.903

Supplemental Table 9) Rates of Mortality or Severe Morbidity Among Patients with Cytokine Levels Above vs. Below Cohort Median

		Mortality or Severe Morbidity in Mild ARDS *(Above vs. Below Cohort Median)*	**Mortality or Severe Morbidity in Moderate ARDS** *(Above vs. Below Cohort Median)*	**Mortality or Severe Morbidity in Severe ARDS** *(Above vs. Below Cohort Median)*
Pro-	**IL-6**	27.3% (09/33) vs. 22.6% (12/53), p=0.627	54.6% (12/22) vs. 28.0% (07/25), p=0.064	64.3% (27/42) vs. 52.6% (10/19), p=0.388
	IL-8	35.7% (10/28) vs. 19.0% (11/58), p=0.090	**57.1% (16/28) vs. 15.8% (03/19), p=0.005**	**70.0% (28/40) vs. 42.9% (09/21), p=0.039**
	IL-18	**35.1% (13/37) vs. 16.3% (08/49), p=0.044**	47.6% (10/21) vs. 34.6% (09/26), p=0.366	**71.8% (28/39) vs. 40.9% (09/22), p=0.018**
	MIP-1β	32.4% (12/37) vs. 18.4% (09/49), p=0.133	54.2% (13/24) vs. 26.1% (06/23), p=0.050	66.7% (24/36) vs. 52.0% (13/25), p=0.249
	TNF-α	31.8% (14/44) vs. 16.7% (07/42), p=0.102	50.0% (14/28) vs. 26.3% (05/19), p=0.104	65.9% (27/41) vs. 50.0% (10/20), p=0.234
Anti-	**IL-1RA**	32.3% (10/31) vs. 20.0% (11/55), p=0.204	50.0% (12/24) vs. 30.4% (07/23), p=0.172	67.5% (27/40) vs. 47.6% (10/21), p=0.131
	IL-10	33.3% (10/30) vs. 19.6% (11/56), p=0.159	**53.9% (14/26) vs. 23.8% (05/21), p=0.037**	**70.3% (29/41) vs. 40.0% (08/20), p=0.021**
	TNF-R2	**38.9% (14/36) vs. 14.0% (07/50), p=0.008**	**56.5% (13/23) vs. 25.0% (06/24), p=0.028**	**75.8% (25/33) vs. 42.9% (12/28), p=0.009**

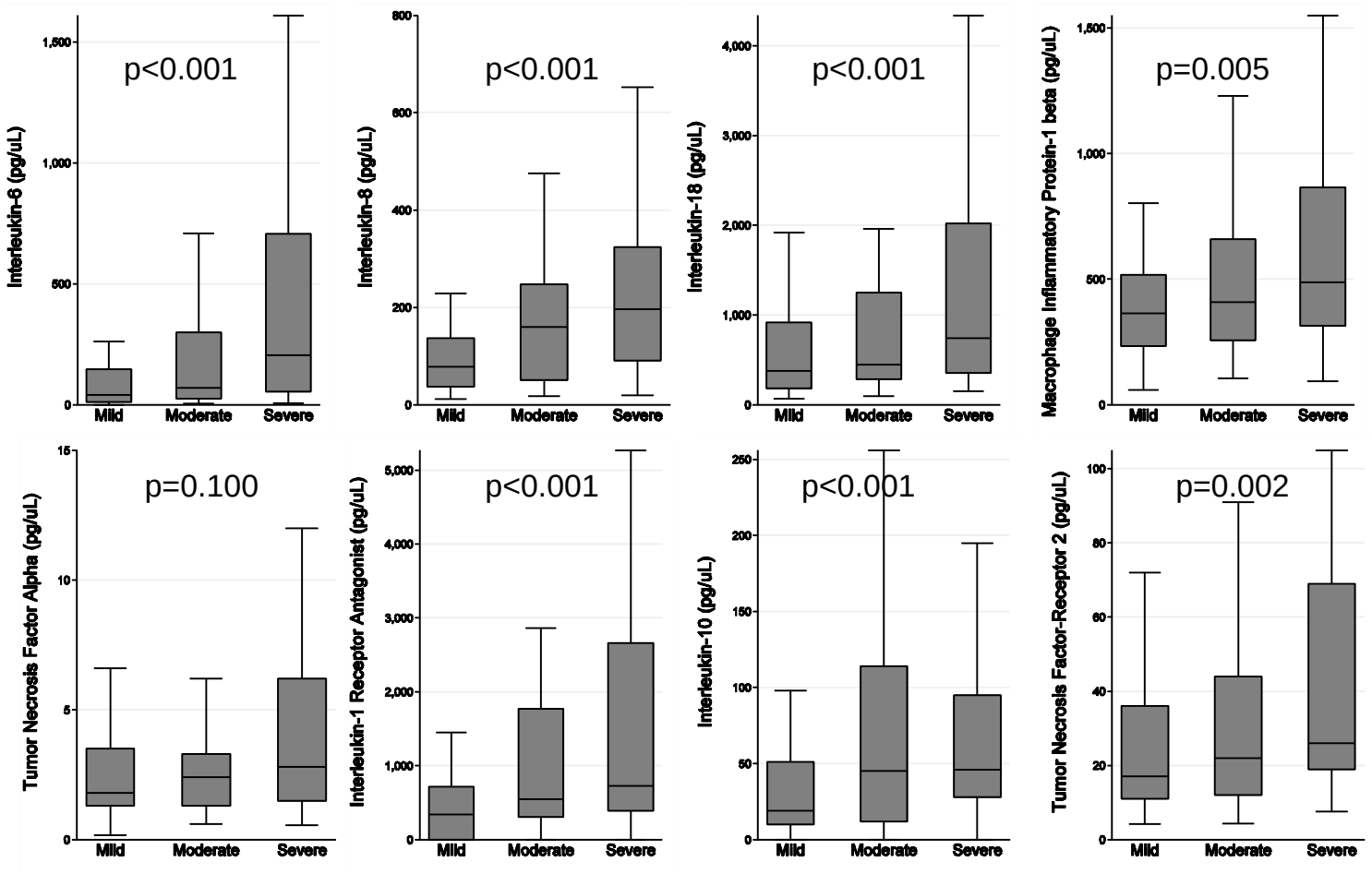
Legend: Pediatric ARDS patients were stratified according to PALICC-defined mild ARDS (4≤OI<8, n=86), moderate ARDS (8≤OI<16, n=47), and severe ARDS (OI≥16, n=61). Patients were then grouped according to whether each measured cytokine level was above vs. below the cohort median. Finally, for each of the 3 ARDS severities and each of the 8 cytokine measurements, the rate of mortality or severe morbidity (top PELOD quartile) was compared for patients with cytokine levels above vs. below the cohort median.

Supplemental Figure 1) A Framework for Understanding Pro- and Anti-Inflammatory Cytokine Networks



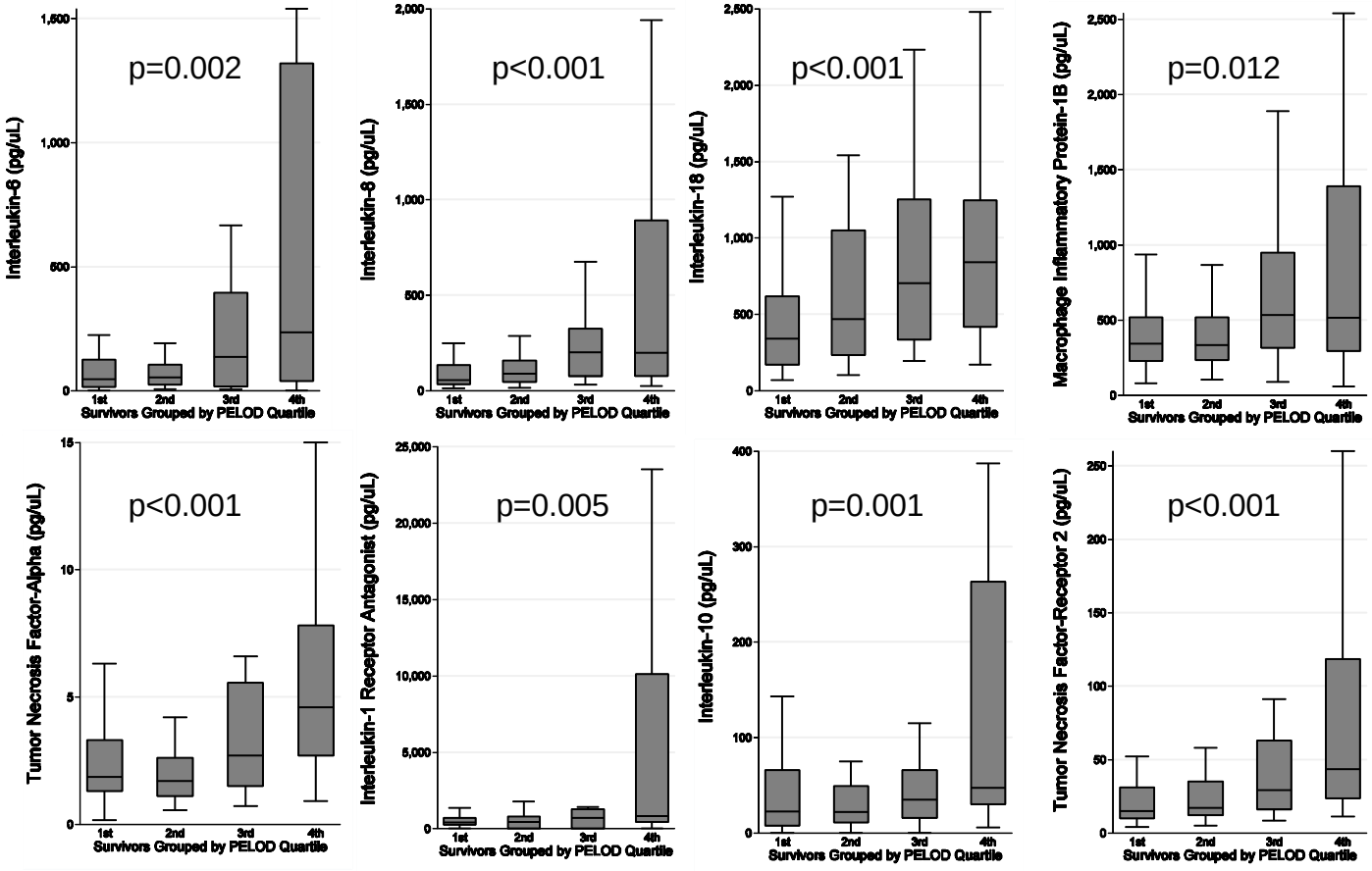
Legend: A reductive list of inflammation-related cytokines mapped according to leukocyte lineage. The majority of pro- and anti-inflammatory cytokines are produced by multiple cell types in response to varying stimuli. Of those shown in this figure, our study includes five pro-inflammatory cytokines (IL-6, IL-8, IL-18, MIP-1β, TNF-α) and three anti-inflammatory cytokines (IL-1RA, IL-10, and TNF-R2).

Supplemental Figure 2) Increasing PALICC Defined ARDS Severity is Associated with Pro- and Anti-Inflammatory Cytokines



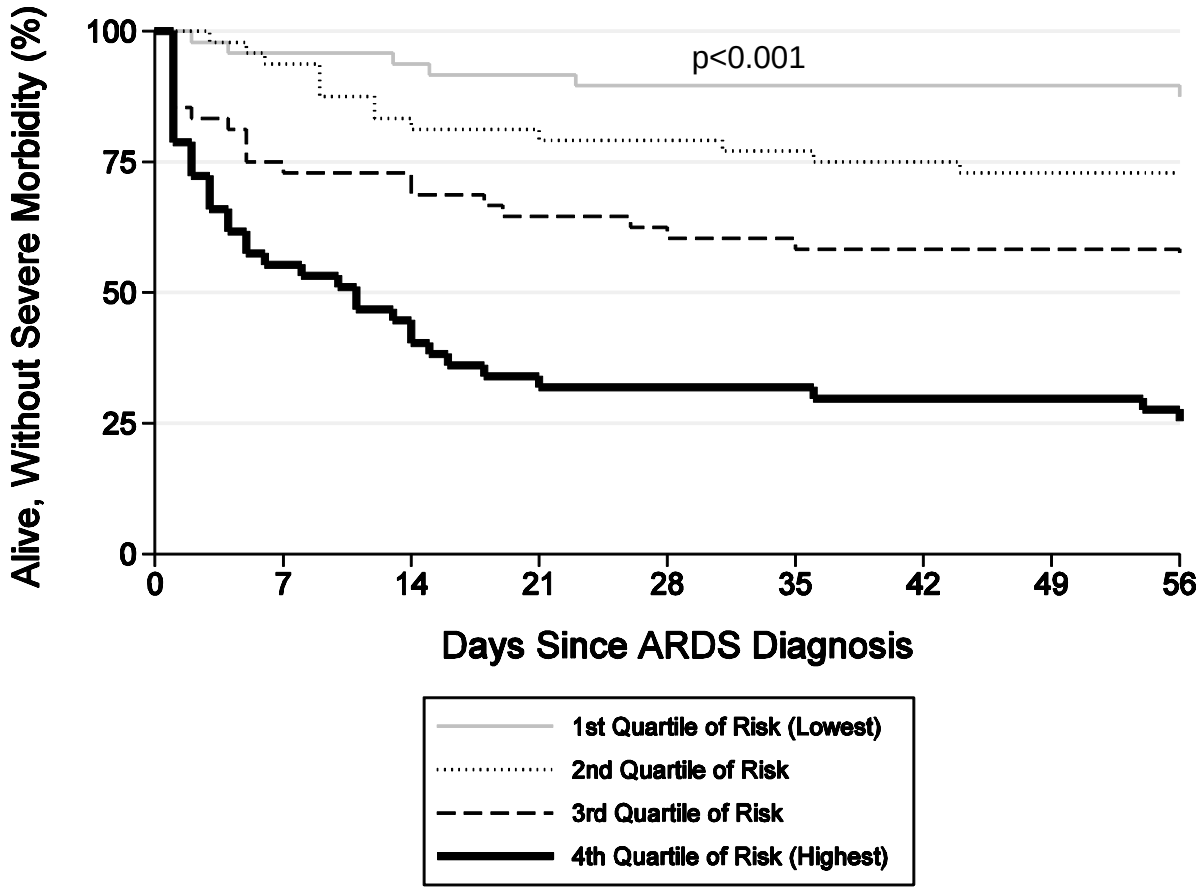
Legend: p-values generated by non-parametric test for trend across ordered groups. PALICC defined mild ARDS includes 4≤OI<8; moderate ARDS includes 8≤OI<16; severe ARDS includes OI≥16.

Supplemental Figure 3) Increasing PELOD is Associated with Pro- and Anti-Inflammatory Cytokines in Pediatric ARDS Survivors



Legend: p-values generated by non-parametric test for trend across ordered groups.

Supplemental Figure 4) Kaplan Meier Function for Time to Death or Severe Morbidity in Pediatric ARDS



Legend: The composite outcome of death or severe morbidity (PELOD≥30) was predicted by the logistic regression of OI, IL-6, IL-8, IL-10, TNF-R2, and HCT history. Patients were then separated into quartile of risk and the Kaplan Meier time-to-event function was plotted. Difference in time-to-event curves was tested by log-rank test of equality and $p<0.001$.

Supplemental References

References

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