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Incorporating Inflammation into Mortality Risk in Pediatric Acute Respiratory Distress Syndrome

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Abstract

Objective—In pediatric ARDS, lung injury is mediated by immune activation and severe inflammation. Therefore, we hypothesized that patients with elevated pro- and anti-inflammatory cytokines would have higher mortality rates and that these biomarkers could improve risk-stratification of poor outcomes.

Design—Multicenter prospective observational study.

Setting—We enrolled patients from 5 academic PICUs between 2008–2015.

Patients—Patients were 1 month to 18 years old, used noninvasive or invasive ventilation, and met the American European Consensus Conference definition of ARDS.

Interventions—None.

Methods—Eight pro-inflammatory and anti-inflammatory cytokines were measured on ARDS day 1 and correlated with mortality, ICU morbidity as measured by survivor PELOD score, and biomarkers of endothelial injury, including angiopoietin-2, von Willebrand Factor, and soluble thrombomodulin.

Measurements & Main Results—We measured biomarker levels in 194 patients, including 38 ARDS nonsurvivors. IL-6, IL-8, IL-10, IL-18, and TNF-R2 were each strongly associated with all-cause mortality, multiple markers of ICU morbidity, and endothelial injury. A multiple logistic

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Author Contributions:

Study concept and design: Zinter, Sapru. *Acquisition of data:* Zinter, Orwoll, Spicer, Alkhoul. *Analysis and interpretation of data:* Zinter, Orwoll, Calfee, Matthay, Sapru. *Drafting of the manuscript:* Zinter. *Critical revision of the manuscript for important intellectual content:* Zinter, Orwoll, Calfee, Matthay, Sapru. *Statistical analysis:* Zinter, Sapru. *Administrative, technical, or material support:* Zinter, Alkhoul, Sapru. *Study supervision:* Sapru. *Approval of final manuscript:* Zinter, Orwoll, Spicer, Alkhoul, Calfee, Matthay, Sapru.

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regression model incorporating OI, IL-8, and TNF-R2 was superior to a model of OI alone in predicting the composite outcome of mortality or severe morbidity (AUROC 0.77 [0.70–0.83] vs. 0.70 [0.62–0.77], $p=0.042$).

Conclusions—In pediatric ARDS, pro- and anti-inflammatory cytokines are strongly associated with mortality, ICU morbidity, and biochemical evidence of endothelial injury. These cytokines significantly improve the ability of the OI to discriminate risk of mortality or severe morbidity and may allow for identification and enrollment of high-risk subgroups for future studies.

Keywords

Intensive care units; cytokines; acute lung injury; hematopoietic stem cell transplantation; interleukins

INTRODUCTION

Pediatric acute respiratory distress syndrome (ARDS) is a clinically heterogeneous disorder characterized by injury to the alveolar-capillary interface, resulting in significant impairment of oxygenation (1, 2). This syndrome is associated with 15–20% mortality as well as substantial morbidity among survivors (3, 4), yet imprecision in identifying high-risk patients early in their course limits the application of experimental therapies that might further improve outcomes. While triggering events include both local and systemic insults, a common pathway is the resultant inflammatory cascade that mediates both pulmonary endothelial and alveolar epithelial damage (5, 6).

A principal component of this inflammatory cascade is the release of chemokines such as IL-8 and macrophage inflammatory protein 1 β (MIP-1 β) by local antigen presenting cells and endothelial cells, leading to migration of activated neutrophils to the lungs (7, 8) and subsequent release of cytokines such as IL-6 and TNF- α (9, 10). In concert, these mechanisms lead to an increase in lung endothelial permeability and ongoing immune activation, which can further propagate the cycle of cell damage (11, 12). We have previously identified evidence of this cell damage in pediatric ARDS patients by correlating mortality with circulating markers of endothelial cell injury, including angiopoietin-2, von Willebrand Factor, and soluble thrombomodulin (13, 14). To date, however, little direct evidence exists linking systemic immune activation with pediatric ARDS outcomes or markers of endothelial damage.

We therefore undertook this study to correlate circulating pro- and anti-inflammatory cytokine levels in pediatric ARDS patients with mortality, morbidity, and biomarkers of endothelial injury. We used a commercially available multiplex panel of 21 cytokines related to macrophage, neutrophil, T-cell, and B-cell activation (Supplemental Figure 1), retained only those reliably detected above the lower limit of quantitation (LLOQ), and then analyzed the performance characteristics of these biomarkers according to consensus guidelines for biomarker development (15–18). We hypothesized that elevated cytokine levels would correlate with clinical outcomes and markers of endothelial damage in pediatric ARDS and might augment the prognostic utility of the OI in predicting mortality and morbidity.

METHODS

Patients

Daily screening occurred at five PICUs between 2008–2015. Inclusion criteria were (1) 30 days < age 18 years, (2) respiratory support of either CPAP, BiPAP, or invasive mechanical ventilation, and (3) ARDS or acute lung injury as defined by the American European Consensus Conference (19). Exclusion criteria were corrected gestational age <36 weeks and limitation of care precluding endotracheal intubation. This study was approved by the Institutional Review Board at each center with consent obtained from each patient/surrogate.

Measurements

All cytokine measurements were carried out on plasma samples collected within 24 hours of onset of ARDS (hereafter referred to as ARDS day 1). As daily pediatric phlebotomy restrictions limited available blood volume for research, we used a multiplex immunoassay to measure IFN- γ , IL-1 α , IL-1 β , IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-23, MIP-1 α , MIP-1 β , TNF- α , and TNF-R2 and an Ultrasensitive Immunoassay to repeat measurement of TNF- α (Myriad RBM, Austin, TX, USA; Supplemental Content). Only cytokines measured above the lower limit of quantitation (LLOQ) in 75% of patients were included in the analysis.

Primary Outcomes

We measured associations between cytokines and two primary outcomes: (1) all-cause hospital mortality and (2) survivor morbidity, estimated by the highest survivor Pediatric Logistic Organ Dysfunction score (PELOD) of measurements taken on days 1–7, 14, 21, and 28 (20). To optimize statistical power for multivariable regression, we combined these two outcomes into a composite outcome of either mortality or severe morbidity, defined as the top quartile of survivor PELOD score.

Secondary Outcomes

We measured associations between cytokines and day 1 ARDS severity, estimated by the PaO₂/FiO₂ ratio (P/F ratio), the oxygenation index (OI), and the Pediatric Risk of Mortality-3 score (PRISM-3), the latter solely in cases where ARDS day 1 coincided with PICU day 0 or 1 (Supplemental Content) (21). When an arterial line was not present, PaO₂ was imputed from SpO₂ 97% according to a log-linear equation (22). Finally, we measured associations between day 1 cytokines and three biomarkers of endothelial injury (Ang-2, vWF, and sTM) (13, 14).

Confounding Variables

To account for underlying variation in cytokine levels due to demographic diversity, we adjusted for age, sex, and patient-reported race (23–25). We also tested for relationships with a history of hematopoietic cellular transplantation (HCT), which we have previously shown to significantly impact pediatric ARDS outcomes and plasma biomarkers (26–28).

Statistics

Selection of variables for logistic regression models used the least angle regression methodology, which employs forward stagewise selection to identify and rank regression variables according to their correlation with outcome, and then maximizes parsimony by identifying among equally robust models the model with the least prediction error (29). Multivariable models were validated using 5-fold internal cross-validation, which minimizes overfitting by deriving the model in a randomly-selected 90% of subjects then repeating the process 5 times and averaging the results (Supplemental Content) (30).

RESULTS

Patient Characteristics and Outcomes

Of 284 enrolled patients, 194 had adequate plasma available for cytokine measurements and were included in this study (Supplemental Table 1). Of these 194, 107 were male (55%), 135 were Caucasian (70%), and the median age was 4.9 years (IQR 0.9–11.5, Table 1). Patients were admitted to the PICU a median of one day before ARDS onset (IQR 0–2) and on the first day after ARDS onset (ARDS day 1), the median P/F ratio was 136.5 (IQR 90.1–218), the median OI was 9.4 (IQR 5.3–18.3), and 176 patients received conventional mechanical ventilation (91%). All-cause hospital mortality occurred in 38 patients (20%) and median survivor PELOD score and ICU LOS were 20 (IQR 11–30) and 12 days (IQR 8–19).

Elevations in Inflammatory Cytokines Are Strongly Associated with Mortality

Five pro-inflammatory cytokines (IL-6, IL-8, IL-18, MIP-1 β , TNF- α) and three anti-inflammatory cytokines (IL-1RA, IL-10, and TNF-R2) were measured above the LLOQ in 75% of patients and were included in subsequent analyses. *Univariate Analysis:* When stratified by mortality, non-survivors had significantly higher levels of IL-6, IL-8, IL-18, IL-10, and TNF-R2 than survivors (Figure 1, Table 2). The AUROCs for discriminating mortality for the 8 cytokines ranged from 0.53–0.68 and was highest for IL-8 (0.68, 95% CI 0.60–0.77); none were significantly different than that of the OI (0.70, 95% CI 0.62–0.77, Supplemental Table 2). *Adjusted Analysis:* In order to account for potential confounders to these relationships, we adjusted for age, sex, race, OI, and history of HCT, the latter being associated with significantly higher levels of multiple inflammatory markers including IL-8, IL-18, and TNF-R2 (Supplemental Tables 3). After adjustment, elevated IL-6 and IL-8 remained significantly associated with increased odds of mortality (Supplemental Table 4). All 8 assessed biomarkers were each positively correlated with the OI and PRISM-3 score, and IL-6, IL-8, IL-18, IL-1RA, IL-10, and TNF-R2 were each negatively correlated with the P/F ratio (Supplemental Table 5, Supplemental Figure 2).

Elevations in Inflammatory Cytokines Are Associated with Survivor ICU Morbidity

To assess morbidity in survivors, we examined the relationships between day 1 cytokines, survivor PELOD score, and ICU LOS. Day 1 IL-6, IL-8, IL-18, MIP-1 β , TNF- α , IL-10, and TNF-R2 were each strongly associated with elevated PELOD score after adjustment for age, sex, and race (Supplemental Table 6). The strongest correlation was observed between PELOD and TNF-R2 ($\rho=0.430$, $p<0.001$). Increasing quartile of survivor PELOD was

significantly associated with greater levels of all cytokines (Supplemental Figure 3). Elevated MIP-1 β and TNF-R2 were weakly associated with increased survivor ICU LOS.

Elevations in Inflammatory Cytokines Are Associated with Biomarkers of Endothelial Injury

In order to associate cytokines with biochemical evidence of endothelial injury, we measured correlations between cytokines and plasma Ang-2, vWF, and sTM. IL-6, IL-8, TNF- α , IL-10, and TNFR-2 were each positively correlated with Ang-2, and IL-8, IL-1RA, IL-10, and TNF-R2 were each positively correlated with sTM (Supplemental Table 7). The strongest correlations were observed between IL-6 and Ang-2 ($\rho=0.463$, $p<0.001$) and between IL-8 and Ang-2 ($\rho=0.443$, $p<0.001$).

Elevations in Inflammatory Cytokines Help Distinguish Risk of Mortality or Severe Morbidity

Given this evidence, we used the least angle regression methodology to select variables for a regression model that would improve risk-stratification of the OI ratio by incorporating biomarker levels. To maximize statistical power, we combined our two primary outcomes into a composite outcome of either mortality or severe morbidity, defined as the top quartile of survivor PELOD score (PELOD ≥ 30). The model with maximum parsimony and minimum prediction error included the OI, IL-8, and TNF-R2 and predicted the composite outcome with a 5-fold internally cross-validated AUROC of 0.77 (95% CI 0.70–0.83). Compared to the OI alone (AUROC 0.70, 95% CI 0.62–0.77), this model had significantly greater fit (likelihood ratio test $p<0.001$) and AUROC ($p=0.042$, Figure 2).

We then used the least angle regression methodology to test an expanded set of variables including the OI, HCT status, any of the 8 measured cytokines, and any of the 3 biomarkers of endothelial injury. The expanded model with maximum parsimony and minimum prediction error included the OI, HCT status, IL-6, IL-8, IL-10, and TNF-R2, predicted mortality or severe morbidity with an internally cross-validated AUROC of 0.79 (95% CI 0.72–0.86) and was well-calibrated over each quartile (Hosmer-Lemeshow goodness-of-fit $p=0.903$, Supplemental Table 8). Compared to the OI alone, this expanded model had significantly greater fit (likelihood ratio test $p<0.001$) and AUROC ($p=0.011$).

When we divided patients into quartiles of risk according to the expanded model, we found that 74% of those in the highest quartile of risk died or had severe morbidity (sensitivity 74%, specificity 72%, likelihood ratio 2.7 [95% CI 2.0–3.7]). This was significantly greater than the 64% rate of death or severe morbidity for those in the top quartile of risk according to the OI alone (sensitivity 64%, specificity 69%; likelihood ratio 2.0 [95% CI 1.5–2.8], McNemar's $p=0.025$). The majority of patients who reached the outcome of death or severe morbidity did so in the first 2 weeks of ICU stay (Supplemental Figure 4).

Elevations in Inflammatory Cytokines Improve PALICC Risk-Stratification

The next goal was to assess whether risk-stratification according to Pediatric Acute Lung Injury Consensus Conference (PALICC) defined ARDS severity could be improved by the addition of cytokine levels (31). ARDS severity on day 1 was strongly associated with both

mortality (10% for mild [9/86], 21% for moderate [10/47], and 31% for severe [19/61], $p=0.007$) and the composite outcome of mortality or severe morbidity (24% for mild [21/86], 40% for moderate [19/47], and 61% for severe [37/61], $p<0.001$). For each of the 3 ARDS severities, we then further stratified patients according to whether their day 1 IL-8 level was above or below the cohort median (116 pg/uL). In both the moderate ARDS group and the severe ARDS group, patients with day 1 IL-8 levels above the cohort median had significantly greater rates of mortality or severe morbidity than patients with day 1 IL-8 levels below the cohort median (57% [16/28] vs. 16% [3/19], $p=0.005$ among those with moderate ARDS; and 70% [28/40] vs. 43% [9/21], $p=0.039$, among those with severe ARDS; Figure 3). We found similar results in analogous comparisons using IL-10 and TNF-R2 (Supplemental Table 9).

DISCUSSION

In this study, we measured plasma levels of pro- and anti-inflammatory cytokines in 194 pediatric ARDS patients. We found a strong relationship between mortality and elevated plasma levels of both pro-inflammatory (IL-6, IL-8, IL-18, MIP-1 β , TNF- α) and anti-inflammatory cytokines (IL-1RA, IL-10, and TNF-R2). These cytokines were also associated with ICU morbidity as measured by survivor PELOD score, early physiologic evidence of lung and multi-organ injury, as evidenced by P/F ratio, OI, and the PRISM-3 score, and biochemical evidence of endothelial injury, including elevated plasma Ang-2 and sTM. Finally, we found that the addition of inflammatory cytokines to the OI improves risk-stratification in a heterogeneous ARDS population.

In terms of significance, this is to our knowledge the largest multicenter study of inflammatory pathways in pediatric ARDS and the first of its size to identify significant early systemic cytokine perturbations in pediatric ARDS nonsurvivors. This important finding builds upon work done in both adult clinical studies and animal models, which have identified alveolar neutrophilic infiltration and cytokine release as hallmark findings of ARDS (10, 32–36). While several studies in pediatrics have associated cytokinemia with meconium aspiration, influenza, and sepsis, this is the first to study a broad range of pro- and anti-inflammatory cytokines in a rigorously defined ARDS framework (37–39).

In terms of prognostic information, we identified significantly improved risk-stratification when OI was combined with day 1 cytokines. For example, among patients with PALICC-defined severe ARDS, those with IL-8 levels above the cohort median had nearly twice the rate of mortality or severe morbidity than those with IL-8 levels below the cohort median ($p=0.039$). Also, when the top quartile of risk according to OI, IL-6, IL-8, IL-10, TNF-R2, and HCT was compared to the top quartile of risk according to OI alone, there was a 15% increase in relative risk for death or severe morbidity (McNemar's $p=0.025$). If applied in a prospective fashion in our cohort, this expanded model would have identified an additional 5 children out of 47 in the top quartile days-to-weeks before the development of death or severe morbidity, allowing for enrollment in research protocols of experimental therapies.

Ultimately, improved risk-stratification is crucial for the identification of candidate high-risk subgroups for the development of new therapeutics, particularly in pediatrics where a major

challenge of clinical trials has been attainment of goal accrual. Once they become widely available, rapid molecular assays could make bedside risk-stratification for clinical trials enrollment a feasible goal and could represent a paradigm shift for pediatric ICU research.

Our study is part of a growing body of pediatric critical care research to use a composite outcome combining mortality and morbidity (40, 41). Neither the mortality rate in our cohort (38/194) nor in that of *Yehya et al* (37/283) (42) is sufficiently high to power a multivariable logistic regression associating mortality with OI and multiple cytokines. Therefore, we generated a composite outcome of mortality or severe morbidity, defined as the top quartile of survivor PELOD (30). This outcome was met by 77/194 patients and facilitated an appropriately powered logistic regression allowing us to show that the composite biomarker of OI, IL-8, and TNF-R2 outperformed the model of OI alone ($p=0.042$). The composite outcome of mortality or severe morbidity is biologically appropriate, clinically relevant, and meets recent PALICC and CPCCRN recommendations for attention to morbidity as a significant PICU outcome (41).

In terms of subgroup phenotyping, we identified higher levels of IL-8, IL-18, IL-10, and TNF-R2 in HCT ARDS patients relative to non-HCT ARDS patients. Recent data suggest that HCT patients may have unique inflammatory derangements after transplantation that might differentiate mortality risk (43, 44). Clinical experience with pulmonary dysfunction during neutrophil engraftment as well as early success of TNF- α inhibition support the exaggerated role of inflammation in this population (45, 46). Although our ability to draw further conclusions was limited by the size of this subgroup, this analysis is an important first step in identifying clinically-validated molecular markers that differentiate subgroups of risk. Further work in identifying unique molecular phenotypes is critical to understanding the pathophysiologic heterogeneity of pediatric ARDS.

Our study has several strengths. First, given the cohort size and diversity of patient characteristics, our results likely have external validity with respect to other academic PICUs. Second, in addition to assessing mortality, we assessed ICU morbidity and biochemical evidence of endothelial injury. The former has been recently emphasized by the PALICC and is particularly important given the lower mortality rates in children in comparison to adults (41, 47), whereas the latter lends biologic and mechanistic plausibility to our findings. Third, we increased our sample size by 15% by imputing PaO₂ from SpO₂ 97% in 26 patients lacking an arterial line but otherwise meeting enrollment criteria.

Our study has some limitations. First, our models were derived in a large pediatric cohort but do require external validation. We attempted to address this by using 5-fold internal cross-validation to minimize error without reducing sample size (30). Second, we did not mandate a uniform ventilator weaning protocol and could not account for variability in ventilator management patterns as they might influence our primary outcomes.

CONCLUSIONS

In conclusion, there was a significant association between mortality and elevated day 1 plasma cytokines in children with ARDS. These cytokines were also associated of ARDS

illness severity, including the P/F ratio, OI, and PRISM-3 score; with ICU morbidity, including survivor ICU LOS and PELOD score; and with biochemical evidence of endothelial injury, including elevated plasma Ang-2 and sTM. These cytokines improve the prognostic performance of the OI in discriminating risk of mortality or severe morbidity and may allow for identification and enrollment of high-risk subgroups for future clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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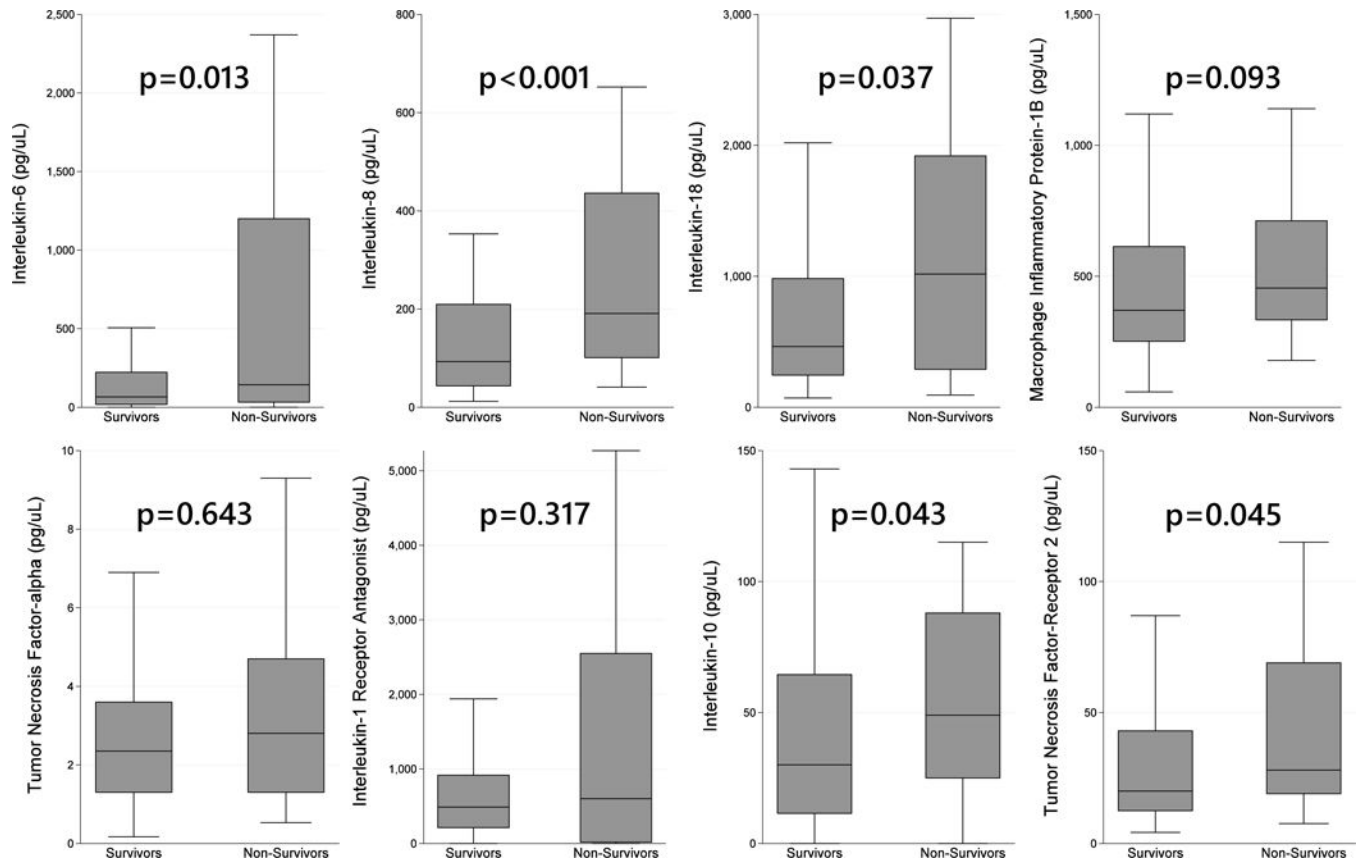


Figure 1. Pro- and Anti-Inflammatory Cytokine Distributions in Pediatric ARDS

Legend: Distributions of pro-inflammatory (IL-6, IL-8, IL-18, MIP-1 β , TNF- α) and anti-inflammatory cytokines (IL-1RA, IL-10, TNF-R2). IL-6, IL-8, IL-18, IL-10, and TNF-R2 were all greater in nonsurvivors than survivors. Upper (75th percentile + 1.5 IQR) and lower (25th percentile – 1.5 IQR) outliers not depicted.

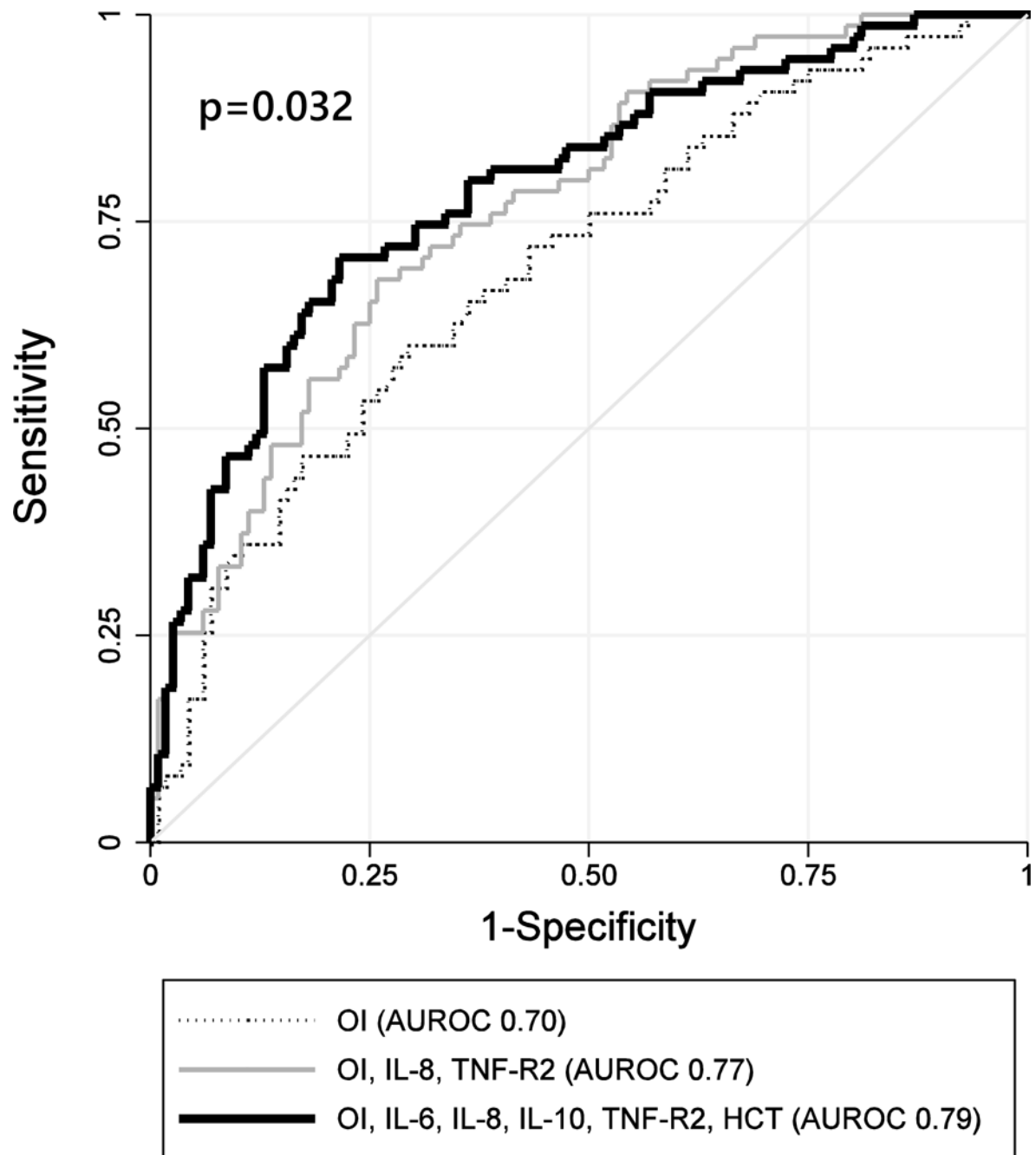


Figure 2. Receiver Operating Characteristics for Mortality or Severe Morbidity in Pediatric ARDS

Legend: The AUROC for the OI ratio (dotted line) was 0.70 (95% CI 0.62–0.77). The AUROC for the model including the OI, IL-8, and TNF-R2 (gray line) was of 0.77 (95% CI 0.70–0.83). The AUROC for the expanded model including the OI, IL-6, IL-8, IL-10, TNF-R2, and HCT history (black line) was of 0.79 (95% CI 0.72–0.86). Chi-squared test for difference in ROC curves $p=0.032$.

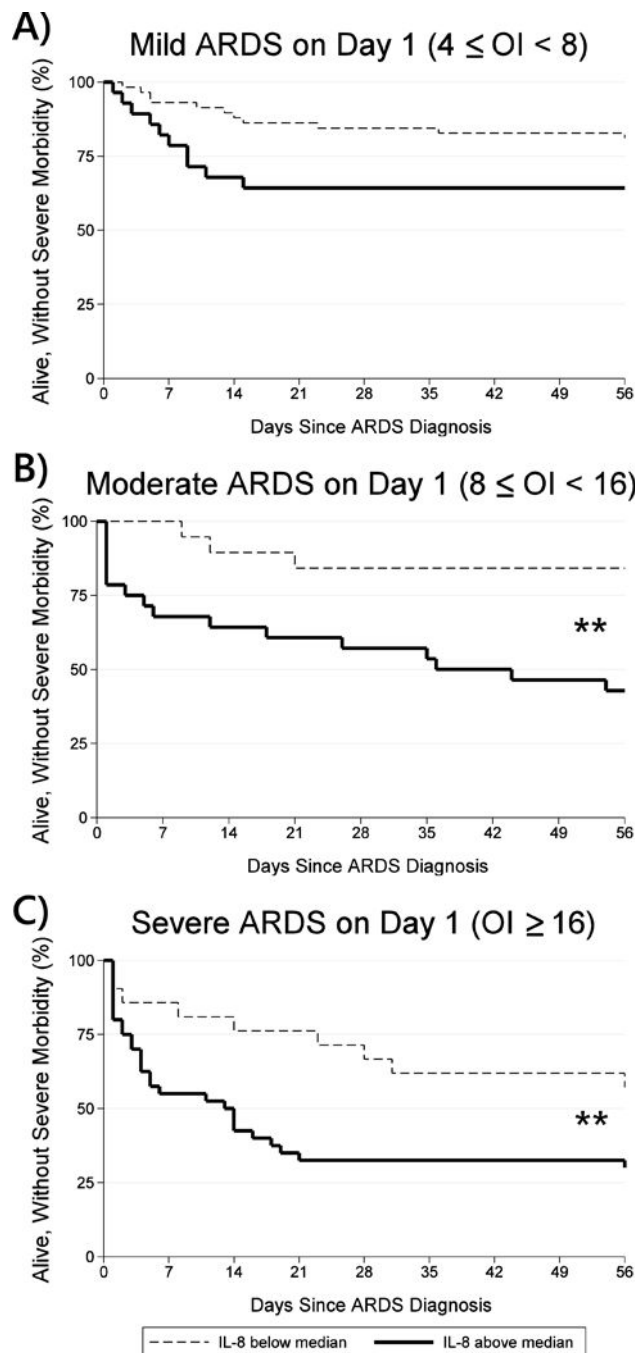


Figure 3. Kaplan Meier Function for Mortality or Severe Morbidity According to PALICC ARDS Severity and IL-8 Level

Legend: **A)** Among patients with mild ARDS ($4 \leq \text{OI} < 8$, $n=86$), mortality or severe morbidity trended towards being more common amongst those with day 1 IL-8 above the median (36%, 10/28) than those below the median (19%, 11/58, $p=0.090$). Log-rank test of equality of Kaplan Meier functions $p=0.061$. **B)** Among patients with moderate ARDS ($8 \leq \text{OI} < 16$, $n=47$), mortality or severe morbidity was more common amongst those with day 1 IL-8 above the median (57%, 16/28) than those below the median (16%, 3/19, $p=0.005$). Log-

rank test of equality of Kaplan Meier functions $p=0.006$. **C)** Among patients with severe ARDS (OI 16, $n=61$), mortality or severe morbidity was more common amongst those with day 1 IL-8 above the median (70%, 28/40) than those below the median (43%, 9/21, $p=0.039$). Log-rank test of equality of Kaplan Meier functions $p=0.023$.

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Table 1
Characteristics of Enrolled Patients with Biomarker Measurements

Legend: Associations tested with Fisher exact test for categorical variables and Wilcoxon rank sum for non-normally distributed continuous variables. P/F ratio n=193 (1 patient excluded due to no arterial line and SpO₂>97%), OI n=191 (1 patient excluded due to no arterial line and SpO₂>97%; 2 patients excluded due to missing MAP), PRISM-3 n=126 (48 patients excluded due to PRISM-3 calculated >1 day before ARDS diagnosis; 20 patients excluded due to majority of PRISM-3 components not measured).

Characteristics	All patients (n=194)	Non-Survivors (n=38)	Survivors (n=156)	Significance
Age (median years, IQR)	4.9 (0.9–11.5)	7.7 (2.5–12.6)	3.8 (0.8–11.4)	p=0.186
Sex (male n, %)	107 (55.2)	25 (65.8)	82 (52.6)	p=0.151
Race (n, %)				p=0.630
White	135 (69.6)	25 (65.8)	110 (70.5)	
Unknown	22 (11.3)	6 (15.8)	16 (10.3)	
Black	14 (7.2)	2 (5.3)	12 (7.7)	
Asian/PI	10 (5.2)	1 (2.6)	9 (5.8)	
Multiple	12 (6.2)	4 (10.5)	8 (5.1)	
American Indian	1 (0.5)	0 (0)	1 (0.6)	
Lung Injury Etiology (n, %)				p=0.619
Pneumonia	109 (56.2)	22 (57.9)	87 (55.8)	
Sepsis	42 (21.7)	9 (23.7)	33 (21.2)	
Other	16 (8.3)	1 (2.6)	15 (9.6)	
Trauma	12 (6.2)	2 (5.3)	10 (6.4)	
Aspiration	10 (5.2)	2 (5.3)	8 (5.1)	
TRALI	5 (2.6)	2 (5.3)	3 (1.9)	
Infectious Trigger (n, %)	151 (77.8)	31 (81.6)	120 (76.9)	p=0.665
HCT (n, %)	21 (10.8)	13 (34.2)	8 (5.1)	p<0.001
Days in PICU Prior to ARDS Onset (median, IQR)	1 (0–2)	1 (0–2)	1 (0–2)	p=0.749
Day 1 Oxygenation Metric (n, %)				p=0.180
Arterial Line (PaO ₂)	168 (86.6)	30 (79.0)	138 (88.5)	
Pulse Oximeter (SpO ₂)	26 (13.4)	8 (21.0)	18 (11.5)	
Day 1 Respiratory Support (n, %)				p=0.808
Noninvasive (CPAP/BiPAP)	9 (4.6)	2 (5.3)	7 (4.5)	
Invasive (Conventional)	176 (90.7)	34 (89.5)	142 (91.0)	
Invasive (HFOV)	9 (4.6)	2 (5.3)	7 (4.5)	
Day 1 Illness Severity (median, IQR)				
PaO ₂ /FiO ₂ Ratio (P/F)	136.5 (90.1–218)	103.8 (85.7–161.7)	146.7 (90.2–226.7)	p=0.027
Oxygenation Index (OI)	9.4 (5.3–18.3)	13.4 (7.8–25.4)	8.0 (5.0–16.9)	p=0.004

Characteristics	All patients (n=194)	Non-Survivors (n=38)	Survivors (n=156)	Significance
PRISM-3	13 (8–21)	18 (12.5–21.5)	13 (7–20)	p=0.009

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Table 2**Plasma Levels of Cytokines, Stratified by Mortality**

Legend: Cytokine levels expressed as median pg/mL with IQR. Association tested with Wilcoxon rank sum test. n=194 for all measurements except n=151 for TNF- α .

Cytokines		All patients (n=194)	Non-Survivors (n=38)	Survivors (n=156)	Significance
Pro-	IL-6	74 (24–264.5)	144 (33–1200)	66 (18–223)	p=0.013
	IL-8	116 (51–236)	191 (101–436)	93 (43.5–209.5)	p<0.001
	IL-18	490 (256–1120)	1017.5 (289–1920)	464 (245–982.5)	p=0.037
	MIP-1 β	390.5 (266–630)	455 (334–712)	370.5 (251.5–613.5)	p=0.093
	TNF- α	2.4 (1.3–3.9)	2.8 (1.3–4.7)	2.4 (1.3–3.6)	p=0.643
Anti-	IL-1RA	544 (186–950)	600.5 (17–2550)	488 (213–915.5)	p=0.317
	IL-10	34 (12–71)	49 (25–88)	30 (11.5–64.5)	p=0.043
	TNF-R2	22 (13–47)	28 (19–69)	20 (12.5–43)	p=0.045